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TRANSACTIONS
OF
THE NEW YORK ACADEMY OF SCIENCES

Editor
ROY WALDO MINER

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Executive Director: EUNICE THOMAS MINER

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REPORT OF THE ANNUAL MEETING

December 2, 1954

The 137th Annual Meeting of the Academy, for the election of Fellows, the presentation of reports, and the transaction of other business, was held at the Academy Building on the evening of Thursday, December 2, 1954.

The first order of business was devoted to the reports of the Officers and Committees of the Academy.

REPORTS OF OFFICERS AND COMMITTEES

Report of the Recording Secretary. The Academy has just completed another year of unusual success and growth. Substantial progress continues to characterize its work in all phases of its activities.

As it will be seen in the Report of the Treasurer, the financial status of the Academy has been improved by more than 33⅓ per cent.

The growth in membership has exceeded that of any previous year in the history of the Academy showing an increase of 15.5 per cent for this year.

It is worthy of note that a total of 306 papers was presented at the meetings of the Academy during the past year, including both Sectional meetings and conferences as compared with a total of 233 papers presented during the preceding year.

In view of this kind of successful development, it is most fitting and a great pleasure to express sincere appreciation and thanks to the Officers and other Members of the Council as well as to the Staff and Members of the Academy at large for their devoted cooperation and ambitious efforts in support of the aims of this remarkable and unique institution serving, as it does, the entire scientific world.

It is a pleasure, again, to thank the Women's Auxiliary for their energetic activity in raising funds toward providing furnishings for the Academy Building. The proceeds obtained from the Bazaars held in 1953 and 1954, and from the Concert held in the spring of 1954 will make possible a complete set of handsome draperies for the West Hall on the first floor of our Building.

Since the establishment of the Societies Administration and Building-Use Division in 1953, the Academy has supervised the business and activities of the Society of Cosmetic Chemists (membership 385), the Veterinary Medical Association of New York City, Inc. (membership 200) and the Association for Computing Machinery (membership 1600). This administration for other societies by the Academy covers such activities as maintenance of financial records, billing for dues and publications, handling of correspondence, processing of new members, printing, preparation and issuance of press releases and arranging for meetings (regular, annual, and conference) as well as for lunches, dinners, and receptions.

Formerly, the business and administration of these societies were handled by their own members located in various parts of the country, this often making the tasks difficult for effectiveness of continuity in administration but, now that the Academy has undertaken this management supervision with centralization of each society's business under the Academy's Executive Director, these organizations are assured of efficient management through the coordinated efforts of an experienced Academy Staff. The results of the Academy's aid in the administration of the business of these three societies have promoted their growth in membership, consolidated their activities, encouraged the expansion of their meetings and publications, as well as effectuated their business management. This is being done on a basis economically beneficial to these societies.

During the past fiscal year, November 1, 1953 to October 31, 1954, three hundred and fifty-one separate meetings were held in the Academy Building. These covered Sectional meetings, conference lunches and dinners, Committee and Council meetings of the Academy, in addition to the meetings of 48 other societies.

The Academy has now become well recognized for its vision and intellectual vigor as exemplified by the quality and number of conferences it holds on special subjects of current research. The wide range of titles dealt with is indicated in the following listing of the twelve conferences held during the past year (an increase of five conferences over those held the previous year):

"Properties of Surfaces," November 5 and 6, 1953, Chairman, Cecil V. King, New York University, New York, N. Y.

"Tissue Culture Technique in Pharmacology," January 14, 15, and 16, 1954, Co-chairmen, C. M. Pomerat, The University of Texas—Medical Branch, Galveston, Texas, and Elmer L. Sevringhaus, Hoffman-La Roche, Inc., Nutley, N. J.

"Recent Advances in the Study of the Structure, Composition and Growth of Mineralized Tissues," January 22 and 23, 1954, Co-chairmen, Roy O. Greep, Harvard School of Dental Medicine, Boston, Mass., and Albert E. Sobel, The Jewish Hospital of Brooklyn, Brooklyn, N. Y.

"Reserpine (Serpasil) and other Alkaloids of *Rauwolfia serpentina:* Chemistry, Pharmacology and Clinical Applications," February 5, 1954, Co-chairmen, Fredrick F. Yonkman and Frank L. Mohr, Research Department, Ciba Pharmaceutical Products, Inc., Summit, N. J.

"The Relation of Immunology to Tissue Homotransplantation," February 12 and 13, 1954, Co-chairmen, John Marquis Converse and Blair O. Rogers, New York University, College of Medicine, New York, N. Y.

"Paul Ehrlich Centennial Conference," March 13, 1954, Co-chairmen, R. J. Schnitzer and E. Grunberg, Hoffmann-La Roche, Inc., Nutley, N. J.

"Ionizing Radiation and the Cell," April 23 and 24, 1954, Chairman, Leslie F. Nims, Brookhaven National Laboratory, Upton, Long Island, N. Y.

"6-Mercaptopurine," April 30 and May 1, 1954, Co-chairmen, George H. Hitchings, Wellcome Research Laboratories, Tuckahoe, N. Y., and C. P. Rhoads, Memorial Hospital Center for Cancer and Allied Diseases, New York, N. Y.

"Leukocytic Functions," (Dedicated to Doctor Hal Downey), May 27, 28, and 29, 1954, Co-chairmen, Albert S. Gordon, Graduate School of Arts and Science, New York University, New York, N. Y.; John W. Rebuck, Henry Ford Hospital, Detroit, Mich.; and Robert S. Speirs, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.

"Speciation and Variation of Asexual Fungi," June 4, 1954, Chairman, Kenneth B. Raper, University of Wisconsin, Madison, Wis.

"Molecular Events in Differentiation Relative to Specificity of Cell Type," October 8 and 9, 1954, Chairman, H. Clark Dalton, New York University, New York, N. Y.

"Instrumentation," October 28 and 29, 1954, Chairman, Joseph Greenspan, Process and Instruments, Brooklyn, N. Y.

The sincere thanks and appreciation of the Academy are hereby extended to the Chairmen of these conferences and to the speakers for the undoubted success of these meetings and the high quality of papers presented.

During this year, 1,769 new Members were added to our rolls as follows: 3 Honorary Life Members; 7 Active Life Members; 1 Corporation Member; 83 Sustaining Members; 1,650 Annual Active Members; and 25 Student Members. Thirty-three former Members were reinstated, thus making a gross total of 1,802 names added to the current Membership rolls.

Four Annual Active Members were transferred to Life Membership, one of whom had paid dues in the Sustaining Class for more than twenty-five years and 36 transfers were effected in the Annual Classes.

The Academy lost by death 4 Honorary Life Members, 4 Active Life Members, 4 Sustaining Members, and 20 Annual Active Members. Three hundred and twenty-one resignations were accepted and 277 Members were dropped as not having paid dues or for not having qualified for Membership.

The Academy now stands with a net gain of 1,171 Members for the current year. There are at present upon the rolls of the Academy, 8,734 Members, of whom 842 are Fellows, classified as follows: 3 Benefactors; 3 Patrons; 63 Honorary Life Members; 386 Active Life Members; 352 Sustaining Members; 7,833 Annual Active Members; 88 Student Members; and 6 Corporation Members.

Record is made with regret of the loss by death of the following Members:

Samuel Barland, elected to Active Membership, 15 December, 1952. Died 27 November, 1953

August Becart, elected to Sustaining Membership, 15 June, 1951. Died August, 1954.
Henry Brand, elected to Active Membership, 24 January, 1952. Died December, 1953.
Moody L. Crowder, elected to Active Membership, 13 August, 1953. Died 13 June, 1954.
Russell W. Davenport, elected to Active Membership, 28 February, 1952. Died 19 April, 1954.
K. George Falk, elected to Active Membership, 1919; transferred to Life Membership, 27 January, 1944. Died 22 November, 1953. Fellow.
Frank R. Ferlaino, elected to Active Membership, 13 May, 1949. Died 2 September, 1953.
Louis W. Haas, elected to Active Membership, 20 February, 1951. Died 28 April, 1953.
Alfred C. Hawkins, elected to Active Membership, 5 December, 1938. Died 30 March, 1954. Fellow.
Rudolf Hober, elected to Active Membership, 22 January, 1948. Died 5 December, 1953. Fellow.
Doyle Joslin, elected to Sustaining Membership, 27 January, 1949. Died 19 August, 1953.
Daniel B. Kirby, elected to Sustaining Membership, 22 October, 1953. Died 27 December, 1953.
Charles H. Lutterloh, elected to Active Membership, 28 April, 1949. Died October, 1953.
Howard C. Moloy, elected to Active Membership, 15 August, 1939. Died March, 1953. Fellow.
Lewis Jefferson Moorman, elected to Active Membership, 17 May, 1954. Died 2 August, 1954.
J. Howard Mueller, elected to Active Membership, 6 October, 1944. Died February, 1954.
Muriel Platt Munro, elected to Active Membership, 15 May, 1947. Died 1953.
Chester N. Myers, elected to Active Membership, 13 June, 1939. Died 3 May, 1954.
Elsie M. B. Naumberg, elected to Active Membership, 1 November, 1920; transferred to Sustaining Membership, 1 January, 1938. Died 25 November, 1953. Fellow.
M. Russell Nelson, elected to Active Membership, 14 July, 1950. Died August, 1954.
Joel B. Peterson, elected to Active Membership, 15 August, 1946. Died December, 1953.
Frank H. Pike, elected to Active Membership, 5 January, 1914; transferred to Life Membership, 4 November, 1935. Died 13 November, 1953. Fellow.
Walter F. Rogers, Jr., elected to Active Membership, 28 February, 1952. Died 18 September, 1953.
Erich Seligmann, elected to Active Membership, 13 September, 1946. Died 1 January, 1954.
Victor Stoll, elected to Active Membership, 27 January, 1949. Died 30 November, 1953.
Harry A. Walker, elected to Active Membership, 23 January, 1947. Died 12 October, 1953.
Curtis L. Weathers, elected to Section Membership, 4 February, 1929; transferred to Active Membership, 28 October, 1937; transferred to Life Membership, 24 February, 1949. Fellow.
Pope Yeatman, elected to Active Membership, 6 November, 1916; transferred to Life Membership, 24 January, 1952. Fellow.

Report of the Corresponding Secretary. There are, at present, upon the rolls of the Academy, 63 Honorary Life Members. The Corresponding Secretary takes this opportunity of presenting Members of the Academy with an up-to-date list of the Honorary Life Membership Class:

Sven Olof Asplund	Gothenburg, Sweden
William Thomas Astbury	Leeds, England
Oswald T. Avery	Nashville, Tennessee
Liberty Hyde Bailey	Ithaca, New York
Ernst David Bergmann	Tel-Aviv, Israel
Charles Herbert Best	Toronto, Canada
Niels Bjerrum	Copenhagen, Denmark
Niels Bohr	Copenhagen, Denmark
Henri Breuil	Paris, France
MacFarlane Burnet	Victoria, Australia
Adolf F. J. Butenandt	Tubingen, Germany
M. Albert Caquot	Paris, France
Alfonso Caso	Mexico D.F., Mexico
Maurice Caullery	Paris, France
James Bertram Collip	London, Canada
Arthur H. Compton	St. Louis, Missouri
Eduardo Cruz-Coke	Santiago, Chile
Henrique Da Rocha Lima	Sao Paulo, Brazil
Henry Dale	London, England
Thomas Dalling	Rome, Italy
Clinton J. Davisson	Charlottesville, Virginia
Peter J. W. Debye	Ithaca, New York
Pere Pierre Teilhard De Chardin	New York, New York
Albert Defant	Innsbruck, Austria
Alexander Fleming	London, England
Howard Walter Florey	Oxford, England
Ross Granville Harrison	New Haven, Connecticut
David Kennedy Henderson	Edinburgh, Scotland
Bernardo A. Houssay	Buenos Aires, Argentina
Julian S. Huxley	London, England
Christopher Kelk Ingold	London, England
Harold Jeffreys	Cambridge, England
Peter Kapitza	Moscow, U.S.S.R.
Arthur Keith	Farnboro, England
Albert Jan Kluyver	Delft, Holland
Alfred L. Kroeber	Berkeley, California
Hugo Rudolph Kruyt	The Hague, Holland
Irving Langmuir	Schenectady, New York

K. S. Lashley	Orange Park, Florida
Kaj. U. Linderstrom-Lang	Copenhagen, Denmark
Robert H. Lowie	Berkeley, California
Vladimir A. Obruchev	Moscow, U.S.S.R.
Marcus L. E. Oliphant	Canberra, Australia
Charles Palache	Charlottesville, Virginia
George H. Parker	Cambridge, Massachusetts
Jean Piaget	Geneva, Switzerland
Henri Pieron	Paris, France
A. R. Radcliffe-Brown	Grahamstown, South Africa
Gaston Leon Ramon	Paris, France
C. G. Rossby	Stockholm, Sweden
John A. M. Runnstrom	Stockholm, Sweden
Henry Norris Russell	Princeton, New Jersey
Erik A. Stensio	Stockholm, Sweden
Th. Svedberg	Uppsala, Sweden
Harald U. Sverdrup	Oslo, Norway
Nicholas H. Swellengrebel	Amsterdam, Holland
Albert Szent-Gyorgyi	Woods Hole, Massachusetts
Arne Tiselius	Uppsala, Sweden
Johannes H. F. Umbgrove	Delft, Holland
Felix A. Vening-Meinesz	Anersfoort, Holland
Max Von Laue	Berlin, Germany
M. S. Watson	London, England
Rudolf Weigl	Cracow, Poland

Record is made with regret of the loss by death of the following Honorary Life Members:

Ludwik Hirszfeld, Wroclaw, Poland, elected 15 December, 1948. Died 7 March, 1954.
Constantin Levaditi, Paris, France, elected 15 December, 1948. Died 5 September, 1953.
Florence R. Sabin, Denver, Colorado, elected 14 December, 1944. Died 3 October, 1953.
Wilhelm Schmidt, Dornach, Switzerland, elected 5 December, 1951. Died 1954.

Report of the Editor. During the fiscal year ending October 31, 1954, The New York Academy of Sciences has published 15 monographs, comprising a total of 2400 pages. Included in these are 225 papers by 336 authors. The pagination of the TRANSACTIONS (440 pages) brings the total pagination issued to 2840 pages. The list of publications issued during the fiscal year is as follows:

ANNALS

Volume 56, Article 6, "The Relation of Lean Body Weight to Metabolism and Some Consequent Systematizations" (1 paper), by A. R. Behnke. Pages 1095-1142. Published November 17, 1953.
Volume 56, Article 7, "Mathematical Deductions from Empirical Relations Between Metabolism, Surface Area, and Weight" (1 paper), by H. von Schelling. Pages 1143-1164. Published April 2, 1954.
Volume 57, Article 2, "The Role of Growth Hormone in Carbohydrate Metabolism" (1 paper), by R. C. de Bodo and M. W. Sinkoff. Pages 23-60. Published June 30, 1953.
Volume 57, Article 3, "Ion Exchange Resins in Medicine and Biological Research" (20 papers), H. Sobotka *et al.* Pages 61-324. Published November 11, 1953.
Volume 57, Article 4, "Branched Molecules" (11 papers), by F. R. Eirich *et al.* Pages 325-450. Published November 19, 1953.
Volume 57, Article 5, "Parental Age and Characteristics of the Offspring" (21 papers), by L. C. Strong *et al.* Pages 451-614. Published January 15, 1954.
Volume 57, Article 6, "Nutritional Factors and Liver Diseases" (34 papers), by K. Schwarz *et al.* Pages 615-962. Published May 10, 1954.
Volume 58, Article 1, "Neurotoxoid Interference with Two Human Strains of Poliomyelitis in Rhesus Monkeys" (1 paper), by M. Sanders, M. G. Soret, and B. A. Akin. Pages 1-12. Published November 30, 1953.
Volume 58, Article 2, "Basic Odor Research Correlation" (27 papers), by A. R. Behnke *et al.* Pages 13-260. Published March 24, 1954.
Volume 58, Article 3, "The Synthesis and Physical-Chemical Properties of New Aromatic Amidines" (1 paper), by M. Kuna and M. J. Kopac. Pages 261-292. Published April 30, 1954.
Volume 58, Article 4, "The Colon: Its Normal and Abnormal Physiology and Therapeutics" (22 papers), by M. L. Tainter *et al.* Pages 293-540. Published July 15, 1954.
Volume 58, Article 5, "The Status of Multiple Sclerosis" (22 papers), by P. Bailey *et al.* Pages 541-720. Published July 28, 1954.
Volume 58, Article 6, "Properties of Surfaces" (15 papers), by C. V. King *et al.* Pages 721-970. Published September 15, 1954.
Volume 59, Article 1, "Reserpine (Serpasil) and Other Alkaloids of *Rauwolfia serpentina:* Chemistry, Pharmacology, and Clinical Applications" (12 papers), by F. F. Yonkman *et al.* Pages 1-140. Published April 30, 1954.
Volume 59, Article 2, "Paul Ehrlich Centennial" (13 papers), by R. J. Schnitzer *et al.* Pages 141-276. Published September 23, 1954.
Volume 60, Article 1, "Speciation and Variation in Asexual Fungi" (15 papers), by K. B. Raper *et al.* Pages 1-182. Published October 29, 1954.

In addition to the articles published above, the following monograph was published during November, 1954:

Volume 58, Article 7, "Tissue Culture Technique in Pharmacology" (28 papers), by C. M. Pomerat *et al.* Pages 971-1326. Published November 17, 1954.

TRANSACTIONS

Series II, Volume 16, Numbers 1-8, consisting of 440 pages, covering the period from November 1953 to June 1954, inclusive, was completed, printed, and distributed during the calendar year.

ANNALS IN PRESS

In addition to the articles published above, the following monographs are now in press:

Volume 59, Article 3, "The Relation of Immunology to Tissue Homotransplantation" (22 papers), by J. M. Converse *et al.* To be published in January 1955.
Volume 59, Article 4, "Ionizing Radiation and the Cell" (16 papers), by L. F. Nims *et al.* To be published about January 1955.
Volume 59, Article 5, "Leukocytic Functions" (28 papers), by A. S. Gordon, J. W. Rebuck, and R. S. Speirs, *et al.* To be published about February 1955.
Volume 60, Article 2, "6-Mercaptopurine" (33 papers), by G. H. Hitchings *et al.* Pages 183-508. To be published December 6, 1954.
Volume 60, Article 3, "New Age Determinations by the Lead Method" (1 paper), by J. L. Kulp, G. L. Bate, and B. J. Geletti. To be published about December 15, 1954.

From November 1, 1953 to October 31, 1954, the Academy distributed 270,246 separate publications and 8,038 complete volumes of the various series as follows:

	Separate Numbers	*Volumes*
Annals		
Members	42,901	
Gratis: Authors, reviews, exchanges and donors	8,545	44
Sales	60,640	77
Authors reprints	88,000	
Transactions		
Members	50,128	
Gratis: Authors, exchanges and reviews	2,436	
Sales	163	137
Authors reprints	10,375	
Special Publications, Volumes I, III, IV		
Gratis: Individuals, reviews		3
Sales		101
Scientific Survey of Porto Rico and the Virgin Islands		
Gratis	3	1
Sales	118	31
Monthly Program		
Members	2,858	7,644
Sales	2,907	
Gratis: Individuals	1,172	
	270,246	8,038

The total *list* value of publications distributed to the members, and gratis to authors, donors, reviewers and exchange institutions is $201,781.33. (Members $170,521.15 — other recipients $31,260.18).

The Editor is pleased to report that the Academy's own printing plant, established in 1953, is not only performing successfully but progressively proving its value. The Printing Division has now been enlarged and is most effective in operation.

An additional press, an ATF No. 20, was installed at the beginning of the year and greatly increased the capacity of the plant, since it prints an eight

page signature on a $14'' \times 20''$ sheet, thus enabling the Academy, on its own premises, to do all of its own miscellaneous printing as well as the Monthly Programs, authors' reprints, short articles in the ANNALS series and especially the TRANSACTIONS. In the less than two years that this press has been in operation, it has not only done all the Academy's printing, except the large conference monographs, with great economy in time and expense, but, through the savings involved, it has recovered the total capital outlay for its equipment and installation, as well as to make possible a considerable expansion of our printing and publishing program.

Report of the Treasurer. The report of the Treasurer presents the financial status of the Academy as of October 31, 1954, and covers the fiscal year of the Academy from November 1, 1953 to October 31, 1954, comprising the Balance Sheet of Assets and Liabilities, in addition to the Statement of Income and Expenses and, in all accounts reflecting the closing figures for that period. These statements of the Balance Sheet clearly summarize the Assets and Liabilities now standing on our Books of Accounting.

The total increase of Assets for this current year amounts to $348,818.33, which is an increase of more than 33⅓ per cent above the closing figures of a year ago.

The most important reason for this increase is due to the bequest of the Academy of the Thomas Lincoln Casey Fund of $213,461.99, received in January, 1954. Following the terms of this bequest, the principal of this Fund has been invested as one of the Endowment Funds of the Academy, with the income being applied to aid in defraying the prime costs of publications in the field of Zoology "in all its departments."

Through the diligent application and effort of the Finance Committee in the reinvestment of the Academy's investment funds, this year, the Reserve Account for Capital Gains and Losses has been increased by $109,365.70, thus bringing this account to a total of $111,603.29.

As a result of these two substantial additions to the Academy's financial resources, the Capital Assets of the Academy, including the Endowment Funds and Prize Funds, the Building Fund and the Academy's Building, now amount to $906,579.01. The current assets for general operations and activities added to the above amount shows the Academy standing, as at October 31, 1954, with complete total assets of $958,147.66. It is hoped that in the year 1955, the Academy will achieve a goal of total standing Assets exceeding one million dollars. We look to the continued support of the Membership and friends of the Academy to aid us in realizing this aim.

In the closing of the Books of Accounting for this fiscal year, the operating surplus account indicates a net General Funds Surplus of $19,869.56 as compared with a General Fund Deficit of $9,451.55 at the beginning of the fiscal year November 1, 1953. Adjustments made with the approval of the auditors in the total amount of $5,888.08 for the Building Fund Capital and Endowment Fund Income were made to the November 1, 1953 Deficit, thus bringing the General Funds Deficit to $15,339.63. The excess of income over expenses for the elapsed fiscal year, therefore, was $35,209.19, as compared with $8,625.24, excess of income over expenses in the previous fiscal year.

Contributions, including grants-in-aid and other cash donations to the work of the Academy totaled $41,945.84. These were given to aid in covering expenses of conferences, travel expenses of speakers, publications, use of the Building and new curtains for the West Hall. The Academy takes this opportunity to express its thanks to these donors for their generous support of its endeavors.

This report of the financial status of the Academy constitutes a record of which we may well be proud, as it now offers continuing promise of operating the Academy on a surplus basis in the years to come, provided we have the coordinated efforts of the Council, Staff and of Membership not only toward this aim but toward the progressive expansion of the Academy's activities.

The Books of Accounting have been checked and certified by the firm of Peat, Marwick, Mitchell and Company, Certified Public Accountants. The property of the Academy has been verified by the Auditors and by the Finance Committee of the Academy.

The report of the Auditors, together with this summary of the Treasurer's Report have been approved by the Finance Committee as of November 29, 1954, as provided in the Bylaws.

Report of the Auditors

PEAT, MARWICK, MITCHELL & CO.
CERTIFIED PUBLIC ACCOUNTANTS
SEVENTY PINE STREET
NEW YORK 5, N. Y.

Accountants' Report

To the Finance Committee
The New York Academy of Sciences
New York, N. Y.

We have examined the balance sheet of The New York Academy of Sciences as of October 31, 1954, and the related statement of General Fund income and expenses and surplus for the year then ended. Our examination was made in accordance with generally accepted auditing standards, and accordingly included such tests of the accounting records and such other auditing procedures as we considered necessary in the circumstances.

The cash and bank balances have been confirmed by count or by certificate obtained directly from the depositary.

The cash held by J. R. Williston, Bruce & Co., carried for the purchase of securities, has been confirmed by acknowledgment from the custodian.

We did not confirm the members' dues or the pledges receivable by communication with the respective members or pledgers. We communicated with all debtors on accounts receivable for publications and on a test basis with those on accounts receivable for use of building, etc. by mailing the Academy's statements for the month of October accompanied by a request that we be notified of any exceptions. The exceptions reported were explained to our satisfaction. Provision has not been made for possible loss in collection of dues, pledges and accounts receivable.

The inventory of the printing division is stated in accordance with a physical inventory taken and valued by employees of the Academy and certified by the Executive Director as to description, quantities, condition and valuation. We observed the inventory taking and checked the prices and computations. The balance sheet does not reflect the books and publications on hand at October 31, 1954 as it is the policy of the Academy to charge publication costs to expense as incurred.

The fund investments were inspected and the income therefrom was confirmed by reference to independent sources.

We inspected the deed to the real estate at 2 East 63rd Street, New York, contributed to the Academy in 1949. It is carried in the balance sheet at the valuation as assessed by the City of New York for real estate tax purposes as of the date of the contribution. The furniture, fixtures and equipment comprise furnishings contributed, to which a value of $10,000.00 was assigned, and purchases to October 31, 1953. The cost of equipment purchased since that date has been charged to the income of the General Fund. In conformity with the practice usually followed by similar organizations, the Academy does not provide for depreciation of the furniture, fixtures, and equipment or of the building.

All liabilities of which we obtained knowledge as a result of our examination have received appropriate recognition.

In our opinion, subject to the foregoing comments, the accompanying balance sheet and statement of General Fund Income and expenses and surplus present fairly the financial position of The New York Academy of Sciences at October 31, 1954 and the results of its operations for the year then ended, on a basis consistent, except as indicated, with that of the preceding year.

/s/Peat, Marwick, Mitchell & Co.

New York, N. Y.
November 26, 1954

THE NEW YORK ACADEMY OF SCIENCES

Balance Sheet
As of October 31, 1954

ASSETS	*Total*	*General Fund*	*Endowment Funds*	*Building Fund*	*Prize Funds*	*Special Funds*
Cash on hand	$ 133.78	$ 133.78				
Cash in The Chase National Bank of the City of New York	30,185.91	502.08	$ 60.68	$ 559.73	$ 1,802.19	$27,261.23
Cash held by J. R. Williston, Bruce & Co.	15,463.73		15,463.73			
Members' dues receivable	5,490.00	5,490.00				
Accounts receivable — sale of publications	8,040.88	8,040.88				
Accounts receivable — use of building, etc.	1,238.50	1,238.50				
Pledges receivable	707.50			707.50		
Prepaid expenses	1,173.92	1,173.92				
Inventory — printing division	5,926.07	5,926.07				
Investments in stocks — stated at purchase cost or quoted price at date of contribution (aggregate quoted market value at October 31, 1954, $467,904.75)	398,917.93		372,962.77	9,408.37	16,546.79	
Real estate, furniture, fixtures and equipment:						
Real estate at 2 East 63rd Street, New York at assessed valuation at date of contribution, and improvements at cost:						
Land	200,000.00			200,000.00		
Building	225,000.00			225,000.00		
Improvements	27,280.63			27,280.63		
Amortization of mortgage paid by General Fund		31,578.13		(31,578.13)		
Furniture, fixtures and equipment — comprising contributed furnishings at assigned value of $10,000.00 and balance at cost (to October 31, 1953)	38,588.81			38,588.81		
Transfers to (from) other funds		(2,000.00)				2,000.00
	$958,147.66	$52,083.36	$388,487.18	$469,966.91	$18,348.98	$29,261.23

Balance Sheet—*Continued*
As of October 31, 1954

LIABILITIES AND FUNDS	*Total*	*General Fund*	*Endowment Funds*	*Building Fund*	*Prize Funds*	*Special Funds*
Accounts payable	$ 8,258.14	$ 8,258.14				
Mortgage (original amount, $200,000.00) on real estate and accrued interest at rate of 4½% (payable $3,750.00 quarterly for principal and interest, and balance due November 1, 1959)	169,685.03	1,263.16		$168,421.87		
Members' dues unearned for calendar year 1954	21,782.50	21,782.50				
Members' dues paid in advance for 1955	910.00	910.00				
Endowment funds:						
John James Audubon Fund	2,500.00		$ 2,500.00			
Nathaniel Lord Britton Fund	29,191.40		29,191.40			
Centennial Endowment Fund	7,934.00		7,934.00			
Publication Fund	3,069.50		3,069.50			
John Strong Newberry Fund	1,000.00		1,000.00			
George H. Sherwood Memorial Fund	7,000.00		7,000.00			
Ralph Winfred Tower Memorial Fund	12,728.00		12,728.00			
Thomas Lincoln Casey Fund	213,461.99		213,461.99			
Capital gains and losses (net) on investment transactions, reserved	111,602.29		111,602.29			
Building fund	301,545.04			301,545.04		
Prize funds:						
George Frederick Kunz Prize Fund	1,247.21				$ 1,247.21	
A. Cressy Morrison Prize Fund	17,101.77				17,101.77	
Special funds:						
Grants-in-aid	21,408.82					$21,408.82
New York Society of Electron Microscopists	4.64					4.64
Contributions:						
Wenner-Gren Foundation prize	1,000.00					1,000.00
Women's Auxiliary	1,095.60					1,095.60
Furniture and fixtures	176.15					176.15
Auditorium project fund	106.27					106.27
Unexpended investment income of endowment funds	5,469.75					5,469.75
General fund surplus, per accompanying statement	19,869.56	19,869.56				
	$958,147.66	$52,083.36	$388,487.18	$469,966.91	$18,348.98	$29,261.23

THE NEW YORK ACADEMY OF SCIENCES

Statement of General Fund Income and Expenses and Surplus

For the Year ended October 31, 1954

Income:		
Membership dues		$124,788.48
Sales of publications		86,704.02
Contributions-in-aid		34,055.99
Contributions—use of building facilities		28,915.92
Contributions to general fund		668.26
Income from securities of endowment and building funds		20,710.91
		295,843.58
Expenses:		
Publication costs (including salaries and expenses of printing division)	$87,742.85	
Distribution of publications	12,270.78	
Postage	13,220.53	
Conferences and sectional meeting expenses	26,051.14	
Building expenses—use of facilities	13,595.16	
Building operating salaries and expenses	22,013.24	
Executive salaries and expenses	12,889.22	
Editorial staff—salaries and expenses	12,633.81	
Membership expansion—salaries and expenses	10,628.82	
Public relations—salaries and expenses	3,986.58	
Annual dinner and meeting expenses	1,568.09	
Societies administration	4,233.32	
Social security taxes	130.09	
Interest on mortgage	7,728.98	
Accounting salaries and expenses	6,994.03	
General office salaries and expenses	8,177.38	243,864.02
Excess of income over expenses for year		51,979.56
Less:		
Cost of equipment purchased	11,300.62	
Unexpended investment income of endowment funds transferred to special funds	5,469.75	16,770.37
General fund surplus for year		35.209.19
Deduct:		
General fund deficit as of October 31, 1953	9,451.55	
Adjustments of other funds applicable to prior years	5,888.08	
		15,339.63
General fund surplus as of October 31, 1954		$ 19,869.56

AWARDS OF PRIZES

(1) *The A. Cressy Morrison Prizes in Natural Science.* After careful consideration and comparison of the eligible papers presented in competition this year, the Committee unanimously awards the prizes as follows:

A prize of $300 to the paper entitled, "The *in vivo* Partition of Manganese among Some Organs and Intracellular Organels of the Rat," by Laurence S. Maynard and George C. Cotzias, Medical Department, Division of Physiology, Brookhaven National Laboratory, Upton, Long Island, New York.

A prize of $300 to the paper entitled, "Drop Density, Weight, Volume, and Time Variations during Chromatographic Column Development," by N. A. Solomon and S. Z. Lewin, Department of Chemistry, New York University, New York, New York.

Honorable mention to the paper entitled, "Effects of Bromide, Nitrate and Iodide on Responses of Skeletal Muscle," by Arthur J. Kahn, Department of

Physiology, Georgetown University Schools of Dentistry, Washington, D. C., and Alexander Sandow, Department of Biology, Washington Square College of Arts and Sciences, New York University, New York, New York.

Honorable mention to the paper entitled, "Olfaction," by L. J. Mullins, Biophysical Laboratory, Purdue University, Lafayette, Indiana.

(2) *The Boris Pregel Prize in the Field of Natural Radioactive Substances.* After careful consideration and after consultation with experts in the field, the Committee unanimously awards this prize of $500 to the paper entitled, "Chlorine-36 in Nature," by Raymond Davis, Jr. and Oliver A. Schaeffer, Chemistry Department, Brookhaven National Laboratory, Upton, Long Island, New York.

Honorable mention is awarded to the paper entitled, "On the Mechanism of Skeletal Fixation of Radium," by William F. Neuman, John B. Hursh, Jean Boyd, and Harold Carpenter Hodge, Division of Pharmacology, Department of Radiation Biology, School of Medicine and Dentistry, University of Rochester, Rochester, New York.

Honorable mention is awarded to the paper entitled, "On the Isotopic Constitution of Radium (Ra-223/Ra-226) in Uranium Minerals, and Recent Problems of Geochronology," by P. K. Kuroda, Department of Chemistry, University of Arkansas, Fayetteville, Arkansas.

(3) *The Wenner-Gren Foundation Prize of the New York Academy of Sciences.* After careful consideration, this prize of $1,000 is awarded to the paper entitled, "The Personality and Motivation of the Research Scientist: A Sociological and Psychometric Investigation," by Raymond B. Cattell, Research Professor, Laboratory of Personality Assessment, Department of Psychology, University of Illinois, Urbana, Illinois.

Honorable mention is awarded to the paper entitled, "The Cell: Some Thoughts on Cytology, Epistemology, and Psychology," by William S. Beck, Atomic Energy Project, University of California, Los Angeles, California.

ELECTION OF FELLOWS AND HONORARY LIFE MEMBERS

The following Members were elected to Fellowship:

Abreu, Benedict E., Ph.D.
Allen, Shannon C., Ph.D.
Anchel, Marjorie, Ph.D.
Anderson, Hamilton H., M.D.
Arrowood, Julia G., M.D.
Bass, Allan D., M.D.
Beers, Yardley, Ph.D.
Behnke, A. R., M.D.
Bender, Max, Ph.D.
Benedetti-Pichler, Anton A., D.Sc.
Berberian, Dicran A., M.D.
Bernheim, Frederick, Ph.D.
Blout, Elkan R., Ph.D.
Blumberg, Harold, D.Sc.
Bondy, Philip Kramer, M.D.
Bonnycastle, Desmond D., M.D.
Boyd, Robert Neilson, Ph.D.
Brunings, Karl J., Ph.D.
Bucher, Nancy L. R., M.D.
Buck, Johannes Sybrandt, Ph.D.
Butt, Edward M., M.D.
Bywater, W. G., Ph.G.
Castellani, Aldo, M.D.
Cavallito, Chester J., Ph.D.
Cefola, Michael, Ph.D.
Clarke, Hans T., D.Sc.
Clinton, Raymond O., Ph.D.
Comroe, J. H., Jr., M.D.
Cortney, Philip, L.L.B.
Coulston, Frederick, Ph.D.
Cranston, Elizabeth M., Ph.D.
Craver, Bradford N., Ph.D.
Croxatto, Hector, M.D.
Cullen, Stuart C., M.D.
Dalton, H. Clark, Ph.D.
Daniels, Troy C., Ph.D.
Del Castillo, Enrique, M.D.
Dennis, Emery Westervelt, Ph.D.
Dounce, Alexander L., Ph.D.
Eaton, Monroe D., M.D.
Elion, Gertrude, M.S.
Engstrom, Arne W., M.D.
Evans, Titus Carr, Ph.D.
Farah, Alfred, Ph.D.
Feirer, William A., M.D.
Fiske, A. Hastings, D.Sc.
Fosbinder, Russell J., Ph.D.
Fox, Sereck H., D. Sc.
Frawley, Thomas Francis, M.D.
Fremont, Rudolph E., M.D.
Garman, Raymond L., Ph.D.
Garnatz, George F., Ch.E.
Gassner, F. X., M.D.V.
Gemmill, Chalmers L., M.D.
Goodhart, Robert Stanley, M.D.
Goulden, Harold D., B.Sc.
Gross, Erwin G., M.D.
Habel, Karl, M.D.
Handley, Carroll A., Ph.D.
Herrmann, Heinz, M.D.
Hoppe, James O., Ph.D.
Houde, Raymond W., M.D.
Jakowska, Sophie, Ph.D.
Jakus, Marie A., Ph.D.
Jensen, H., Ph.D.,
Jorpes, Johan E., M.D.
Kade, Charles F., Jr., Ph.D.
Karnofsky, David A., M.D.
Kirchner, Fred K., Ph.D.
Kisch, Bruno, M.D.
Kleiderer, Ervin C., Ph.D.
Knisely, Melvin H., Ph.D.
Koelle, George B., Ph.D.
Krantz, John C., Jr., Ph.D.
Langlykke, Asger F., Ph.D.
Lee, James W., D.D.S.
Lehman, Arnold J., M.D.
Lehman, Robert A., Ph.D.

Lehr, David, M.D.
Lesuk, Alex, Ph.D.
Leuallen, E. Emerson, D.Sc.
Lewis, John R., Ph.D.
Loew, Earl R., Ph.D.
Looney, Joseph M., M.D.
Lucia, S. P., D.Sc.
Luduena, F. P., M.D.
Maramorosch, Karl, Ph.D.
Markee, J.E., Ph.D.
Marple, Charles Dalliba, M.D.
Martell, Arthur E., Ph.D.
Marti-Ibanez, Felix, M.D.
Melville, Kenneth Ivan, M.D.
Menotti, Amel R., Ph.D.
Moore, Dan H., Ph.D.
Moore, Francis D., M.D.
Moore, Maurice L., Ph.D.
O'Leary, Paul Arthur, M.D.
Orth, O.S., M.D.
Ott, Emil, D.Sc.
Palade, George E., M.D.
Pfeiffer, Carl C., Ph.D.
Pomerat, Charles Marc, Ph.D.
Quastel, J. H., D.Sc.
Quigley, J. P., Ph.D.
Ratner, Sarah, Ph.D.
Raymond, Albert L., Ph.D.
Reifenstein, Edward C., Jr., M.D.
Richardson, Arthur P., M.D.
Ruddy, A. Wayne, Ph.D.
Rudzinska, Maria A., Ph.D.
Rutstein, David D., M.D.
Shamos, Morris H., Ph.D.
Sherndal, A. E., D.Sc.
Sondern, Clarence W., Ph.D.
Summerson, William H., Ph.D.
Sweet, Leon A., Ph.D.
Tahmisian, Theodore Newton, Ph.D.
Tarr, Leonard, M.D.
Taylor, T. Ivan, Ph.D.
Thompson, Randall L., M.D.
Travell, Janet, M.D.
Turnbull, David, Ph.D.
Warner, Robert C., Ph.D.
Weaver, Harry M., Ph.D.
Wegria, Rene, D.Sc.
Winsor, Travis, M.D.
Wood, Paul M., M.D.
Zettlemoyer, Albert C., Ph.D.

Honorary Life Membership was conferred upon the following eminent scientists:

Ulich R. Evans, University of Cambridge, Cambridge, England.
Emmanuel Faure-Fremiet, College de France, Paris, France.

ELECTION OF OFFICERS

Report of the Tellers. The Tellers appointed by the President to count the ballots for the Elected Officers and other Members of the Council to serve for the year 1955 are pleased to report that, by the 1,842 ballots returned from the Membership, the official slate, as nominated by the Council, was overwhelmingly elected.

The list thus chosen by the Membership of the Academy for the year 1955 is as follows:

President-Elect
WALTER S. ROOT

Vice-Presidents
WILLIAM H. COLE — ROSS F. NIGRELLI

Recording Secretary
CHARLES W. MUSHETT

Corresponding Secretary
JUNIUS BIRD

Treasurer
RICHARD O. ROBLIN

Editor
ROY WALDO MINER

Councilors (1955-1957)
M. J. KOPAC — LLOYD C. MILLER
C. P. RHOADS — ELMER M. SEVRINGHAUS

Finance Committee
HARDEN F. TAYLOR, *Chairman*
GORDON Y. BILLARD — ROBERT F. LIGHT

Chairmen of Sections
ANGELINA MESSINA, *Geology and Mineralogy*
HILARY KOPROWSKI, *Biology*
ALBERTA S. GILINSKY, *Psychology*
JOSEPH BRAM, *Anthropology*
CECIL V. KING, *Physics and Chemistry*
ERNEST J. CHRISTIE, *Oceanography and Meteorology*
NICHOLAS V. FEODOROFF, *Mathematics and Engineering*

Chairman of Division
JOHN B. ROUTIEN, *Mycology*

PROGRAM OF THE EVENING

After the Business Meeting the following program was given:
Research in a Zoological Park. **John Tee-Van, Retiring President, The New York Academy of Sciences.**
The Gentlemen Scientists of Old New York. **Chauncey D. Leake, Executive Director, The University of Texas—Medical Branch, Galveston, Texas.**

RESEARCH IN A ZOOLOGICAL PARK

By John Tee-Van

Retiring President, The New York Academy of Sciences,
Director, New York Zoological Society

I wish to discuss briefly the development of research at a zoological park and an aquarium, with special reference to the New York Zoological Park, otherwise known as the "Bronx Zoo." In the short time at my disposal, I can give you but a brief insight into the many fascinating fields of endeavor offered in a zoological park. Few people realize that research is carried on in a place where the normal operation is to exhibit animals. They think of a combined park and aquarium as a place where they may come to look at animals, to have meals at the restaurants, and to participate in the recreation offered by a park. This conception is valid, for the most part, of zoos the world over, for the few organizations among them that participate in research can be counted on the fingers of one hand. A few European zoos do spasmodically produce research, and, in America, the number of such zoos is still smaller.

Possibly, the fact that we publish a technical journal, *Zoologica*, may have something to do with our research activities, although it may be a question as to whether the cart is before the horse or the horse before the cart. Under any circumstances, the New York Zoo and the London zoos are the principal institutions of their kind to have their own technical scientific journals. The London Zoo has its *Proceedings* and its *Transactions*, publications that are principally produced by workers outside the institutions that have developed them. We have *Zoologica*.

Research in our Zoological Park dates back almost to the beginning of the park in 1899. During the first few years, while the park was being developed and generally organized, technical reports were issued as part of the *Annual Report;* but, in 1907, the magazine, *Zoologica*, was established and continues to this day as a quarterly publication.

Among the earliest research workers at the Zoological Park was Doctor William Beebe, one of those honored here this evening. In fact, he was working at the park on the day of its opening in 1899. Although now retired, he continues his work to the present day. At 77 years of age, he is still active. Only a few weeks ago he left New York, on the 53rd ex-

pedition of his department, for Simla, Trinidad, where the society's tropical laboratory is established, though his basic laboratory and center of operations is at the Park, in New York. These 53 expeditions have been comprehensive in scope and world-wide in geographic extent. An expedition to Asia, during the period from 1909 to 1911, resulted in the four-volume "Monograph of the Pheasants," which received the Elliott Medal of the National Academy of Sciences. After 1916, Doctor Beebe's work was established as a separate department of the Society, the Department of Tropical Research, as Director of which he has labored in British Guiana, Venezuela, the Galapagos Islands, Trinidad, the Sargasso Sea, and also Bermuda, where his bathysphere descents climaxed the intensive work prosecuted on coastal and deep sea fishes. In 1953, the publications issued by his department alone totaled 943. At the present time, Doctor Beebe and his associates are working on a monograph of the life histories of a group of moths, the Euchromidae, with special relation to their behavior.

Miss Jocelyn Crane, Assistant Director of the department, is an expert on deep sea fish, fiddler crabs, and especially on butterflies. She is now working on the morphology, ecology, and behavior of a single species of fiddler crab which ranges from Cape Cod to Rio de Janeiro. She is also doing experimental work in the insectaries of the Society's Trinidad field station, where she is dealing with the comparative behavior of two species of red-and-black *Heliconius* butterflies. Other field work on butterflies is expected to shed light on the phylogeny of these insects and on the organization of insect behavior.

I, too, worked in Doctor Beebe's department for 25 years, until 1941, on the marine fish of the western North Atlantic and the eastern Pacific, culminating the work by becoming editor-in-chief of *Fishes of the Western North Atlantic*. One 576-page volume of this publication has already appeared, and the second volume, of 588 pages, will be issued within a few days.

After the renovation of the Reptile House of the Zoological Park into the first-ranking reptile house in the world, Doctor James A. Oliver became busily engaged on research in reptiles, principally snakes. Particular attention is being paid to temperature physiology in connection with the special differentiation of snakes in temperature selection, when placed in the variable temperature box, and also on the effect of temperature on production capacity, growth, and longevity. A small amount of work on venomous snakes and snake venoms is also being done with the cooperation of other institutions.

In 1947, a group of four summer fellowships on animal behavior was established at the Zoological Park. This work continued with different Fellows for a number of summers and resulted in studies on the great apes, penguins, hummingbirds, and the elephants; also on investigations

in herd differentiation in the ungulates, and other similar activities.

Doctor John Quaranta studied the ability of the Galapagos and Aldabra giant tortoises to determine color differences, devising complicated electrical apparatus to enable him to confront these animals with differently colored glasses.

Recently, Doctor Quaranta, together with A. J. Summor, has been studying the age differences in the perceptual responses of the aquatic fowl to three-dimensional geometric designs. The influence of social isolation on the perceptual responses of the aquatic fowl and the influence of auditory deprivation and visual deprivation upon the emotional responses of aquatic fowl, as measured by the psychogalvanometer, were also investigated. In addition, the perceptual responses of aquatic fowl to figure-ground configurations were investigated. The work in this field is limitless.

Lee S. Crandall, General Curator Emeritus of the New York Zoological Park, is concerned with research on the maintenance, breeding, and exhibition of mammals and birds in captivity. At the present time, he is engaged in recording the results of this research in volume form for publication. The principal aspects that are being developed are those relating to diet, longevity, breeding, and management in relation to advantageous presentation to the public. General natural history and animal psychology are being considered only in so far as they bear on the basic thesis.

The work of the two veterinarians of the Zoological Park, Doctor Leonard J. Goss, Assistant Director and Doctor Charles P. Gandal, in addition to the multitudinous tasks involved in the care of the animals, includes quantitative and qualitative hematology studies on specimens as they are presented for clinical treatment. The purpose of this work is to have, ultimately, a sufficient number of determinations to establish the normal blood counts of various species.

Certain investigations in veterinary clinical research are continuously in progress in the animal hospital. These include such matters as interspecies blood transfusions, intramedullary metal pins for repairing bone fractures in birds, and applications of various drug and hormonal preparations in wild animals.

The phases of research that the hospital has been working on, in cooperation with other institutions, are as follows: blood serum for comparative serology has been sent to the Serological Museum at Rutgers University; blood from various species for linolenic acid determination has been obtained for the Bronx Hospital; turtle blood has been developed for the Messinger Research Laboratories at Beth-El Hospital in Brooklyn for a study relating to arteriosclerosis; and, finally, blood for electrophoretic studies has been devised for the New York State Psychiatric Institute.

The group of workers in the aquarium, four in number, has been able, during the past 13 years, to devote more time to research than will be possible in the immediate future, due to the demolition of the old Aquarium at Battery Park. This is especially true of Christopher W. Coates and James W. Atz, Curator and Assistant Curator, respectively, of the Aquarium. With the new Aquarium at Coney Island now under way, these two members of the staff will have less time to devote to research.

Mr. Coates's dominant research activities still center about the electric eel and the surface configurations of the chemical components of the nerve cells of the animal. This work is financed almost entirely from outside, except for our contributions in the matter of procurement, food, care, and a few relatively minor expenditures by various governmental agencies, including the Atomic Energy Commission, Department of the Army, Office of Naval Research, and Public Health Service. Each of these groups seeds the answers to specific questions within its own interest, but each, also, shares in the support to general fundamental research.

As side products, Mr. Coates and his group have recently discovered two new species of electric fishes, *Eigenmannia virescens* and *Gymnorhamphichthys hypostomus*, each of which, apparently, uses its electricity for the purpose of finding its way about and not for either defense or offense, as do the better known electric fishes. This discovery ties in with the previously developed ideas that the electric eel has a sort of "radar" which it uses to locate food objects and to find its way about generally.

Mr. Atz and Doctor Grace E. Pickford of the Bingham Oceanographic Laboratory of Yale University are writing an analytical review of what is known about the pituitary physiology of fishes. This review should prove of value not only to ichthyologists and physiologists, but also to fish culturists and conservationists, since reproduction under controlled conditions can be accomplished by the use of pituitary injections.

As a doctoral thesis, Mr. Atz, under the guidance of Doctor Myron Gordon, is studying the genetics and evolution of some of the platyfishes of Mexico. The problems of polymorphism and gene homology figure prominently in this study, which will include genetical analyses as well as morphological and ecological comparisons.

Doctor Gordon's work in the genetics of fishes has led to the melanomas, or black cancers. The crosses that he has bred between platyfishes and swordtails show disturbances in the black pigment cells which, in the selected crosses, develop into melanomas.

The techniques of genetics, mathematics, ecology, histology, pathology, regeneration, and transplantation have been joined in an effort to understand the intricate interplay of the forces underlying the spontaneous transformation of normal pigment and thyroid cells into neoplastic cells.

The basic aim of Gordon's research is to determine how the development of these neoplasms is linked to specific genes or, more likely, to a constellation of genes which favor atypical cell growth.

Modern theories relating to physiological and population genetics have been applied to the problem of normal and atypical pigment cell growth. Variously pigmented fishes from geographically isolated populations were obtained and intermated. The growth of their large, sensitive pigment cells was exaggerated in some intraspecific hybrids and was suppressed in others. Proof has recently been obtained of the transformation of macromelanophores into melanocytes, the key cells of the melanoma.

We come next to the work of the Recording Secretary of the Academy, Doctor Ross Nigrelli. He and his colleagues in many institutions have worked on the parasites and metabolic diseases in captive fishes. They have investigated the role of diseases, especially those caused by microorganisms, on fish catastrophes caused by natural fluctuations of physical and chemical factors of water and by pollution; on the imbalance of the biocenose as cause of natural mortalities; on the phenomenon of antibiosis among aquatic invertebrates; on the isolation of pharmacologically active agents from marine organisms; and on the comparative pathology of neoplastic and other tumors.

Doctor Nigrelli's present researches are concerned with the following subjects: the histopathological and hematological recognition of diseases in fishes; the role of viruses and rickettsial-like organisms as etiological agents in fish diseases, especially in the development of tumors and other atypical cell growths in fishes; the isolation and cultivation of certain pathogenic bacteria and protozoa; and the isolation of antibiotic substances and other pharmacologically active agents from marine organisms.

Thus, we can see that research at a zoological park and aquarium may be the work of many individuals on many diverse subjects, the entire product forming a considerable accretion of scientific data. The fact that the New York Zoological Society's President, Fairfield Osborn, is here, this evening, is an indication of the interest displayed by the Society in research and its involvements. Research in a zoological park is a many-facetted affair, and its results blaze forth in many directions.

President Tee-Van then exhibited an interesting and comprehensive motion picture, illustrating vividly many of the phases of the research on wild animals in captivity, as carried on in the New York Zoological Park.

THE GENTLEMEN SCIENTISTS OF OLD NEW YORK*

By Chauncey D. Leake

Executive Director, The University of Texas - Medical Branch
Galveston, Texas

(Abstract)

Old New York must have been a delightfully stimulating intellectual community at the close of the Napoleonic wars when The New York Academy of Sciences was established. This was the big day for a brilliant group of friends gathered together for the exchange of curious bits of "useful information," including many original observations and often hitherto unknown facts. Many of these friends were physicians. All of them were true lovers of scientific pursuits, and significant natural scientists in their own direct contributions in medicine, agriculture, chemistry, botany, mineralogy, and geology. Many of them had been trained in Edinburgh and brought the steady Scotch spirit of independent curiosity and of freedom with responsibility to their mutual work. Included in this group were Samuel Bard (1742-1821), Samuel Latham Mitchill (1764-1831), and David Hosack (1769-1835). These were the physicians who started medical training and research in old New York. Their close friend was DeWitt Clinton (1769-1828) who was an excellent practical agriculturist, sanitarian, and political scientist. There were the amazing Beck Brothers (John B., 1794-1851; Lewis C., 1798-1853; and Theodor Romeyn, 1791-1855), who contributed so many reports on natural history, geology, and medical jurisprudence. The attractiveness of New York as a scientific center was strong enough to bring James F. Dana (1793-1827) to the group and his brilliant lectures on chemistry and electricity stimulated the practical work of Samuel F. B. Morse (1791-1872) to turn from art to the telegraph.

These gentlemen scientists of old New York established medical schools, medical and scientific journals, and medical and scientific societies.

They maintained museum collections and developed botanical gardens. They set the standard for far-seeing public interest in scientific affairs which has become a permanent characteristic of the enlightened people in the greatest city in the world today.

*It is hoped that the full article by Doctor Leake will be published in a later issue.

ABSTRACTS OF PRIZE-WINNING PAPERS

CHLORINE-36 IN NATURE

By Raymond Davis, Jr., and Oliver A. Schaeffer*

Awarded the Boris Pregel Prize in Natural Radioactive Substances, 1954

The 308,000-year half life chlorine isotope, chlorine-36, has been detected in nature. Estimates were made of the amounts of chlorine-36 radioactivity induced by cosmic ray neutrons in surface rocks and various saline waters. The analysis showed that measurable amounts should be present in certain surface rocks containing chlorine.

Measurements were made by low level counting techniques on chlorine isolated from four natural sources: a phonolite rock from Cripple Creek in Colorado; a syenite rock from Red Hill in New Hampshire; Great Salt Lake water; and Atlantic Ocean water. The phonolite contained 0.37± .04 disintegrations per minute per gram of chlorine, and the other samples contained less activity than the minimum detectable with the counter used.

A discussion is given of the possibility of employing the rate of growth of chlorine-36 in surface rocks as a means of establishing a time scale for geological events in the range 60,000 years to 1,000,000 years ago.

DROP DENSITY, WEIGHT, VOLUME, AND TIME VARIATIONS DURING CHROMATOGRAPHIC COLUMN DEVELOPMENT

By N. A. Solomon and S. Z. Lewin**

Awarded an A. Cressy Morrison Prize in Natural Science, 1954

The problem of the development of rational fraction-cutting procedures in column chromatography based upon effluent composition is attacked in a novel way by investigating the nature and magnitude of the variations in the drop-times, weights, volumes, and densities of effluent drops forming freely at the base of a chromatographic column. Elution and frontal developments are investigated with the active adsorbents alumina and charcoal, and with the inert material, silicon carbide. The solvents used are water, Rochelle salt solution, sodium chloride solution, and aqueous acetone.

It is shown that the hitherto unexploited properties of drop-time, weight, and volume show relatively large changes during the course of the chromatogram, and that these effects coincide with changes in the composition of the effluent. The drop weight variations are larger than those to be expected on the basis of surface tension effects, and it is concluded that the drop-time markedly affects the drop weight.

*Chemistry Department, Brookhaven National Laboratory, Upton, Long Island, N. Y.
**Department of Chemistry, New York University, New York, N. Y.

The drop-time variations for carborundum columns are in good agreement with deductions from the equations for viscous flow. However, with alumina columns these variations are far too large to be accounted for solely by viscous flow. It is concluded that for alumina columns the streaming potentials existing at the solid-effluent interfaces are the controlling factors.

It is shown that for many systems the drop-time is a very sensitive and convenient indicator of effluent composition, and its application to column chromatography with colorless solutes offers great promise.

THE *IN VIVO* PARTITION OF MANGANESE AMONG SOME ORGANS AND INTRACELLULAR ORGANELS OF THE RAT

By Laurence S. Maynard and George C. Cotzias*

Awarded an A. Cressy Morrison Prize in Natural Science, 1954

The essential metabolic role of manganese in the animal organism has long been a matter of record. It was an early finding that intravenously injected manganous salts are removed quickly from the bloodstream, leaving the body somewhat less rapidly by way of the biliary tract. In the course of studies of the kinetics of manganese, our observations led us to surmise the existence in the body of a mechanism whereby the manganese is temporarily diverted in transit, entrapped, and then gradually released. Such a mechanism, with the resultant rapid movement of the element into the entrapping system, would provide an explanation for the lack of agreement noted in previous studies of this metal's organ distribution.

The isotope used in this study, manganese, has the advantage of being readily available to us in sufficiently high specific activity that dosages need not exceed levels of normal tolerance. The short half life of this material confined our attention to the early portion of the distribution process before reabsorption and redistribution of the isotope become a factor to confuse interpretation of the results.

From organ distribution studies, a correlation became apparent between the richness in mitochondria of a given organ and its ability to concentrate manganese. We therefore extended our studies to include the mitochondrial uptake of this element *in vivo* in order to determine whether these organels were a preferential site of manganese accumulation and, presumably, of its metabolic activity.

(1) Intraperitoneally injected radioactive manganese (Mn56) is rapidly distributed, concentrating primarily in organs rich in mitochondria.

(2) Liver cell fractionation studies confirm that the mitochondria are the principal intracellular site of manganese uptake.

(3) The observed distribution of manganese would be compatible with its functioning as a respiratory co-factor.

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THE PERSONALITY AND MOTIVATION OF THE RESEARCH SCIENTIST: A SOCIOLOGICAL AND PSYCHOMETRIC INVESTIGATION

By Raymond B. Cattell*

Awarded The Wenner-Gren Foundation Prize of The New York Academy of Sciences, 1954

When Sir Humphrey Davy, toward the end of a life made illustrious by great discoveries, was asked to name his greatest, he replied, "My best discovery was Michael Faraday." We may smile, with mixed thoughts, over this famous remark, but, in fact, its graciousness is exceeded by its very truth. Whatever Sir Humphrey may have thought privately about his greatest discoveries, no one of them did so much for science as the time he put into encouraging and stimulating his apprentice, Michael Faraday.

Like any other researcher of mature years, I can similarly record that some of the greatest pleasure in research has arisen from the spectacle of my two or three most brilliant students over that period taking the bone of discovery in their teeth and vigorously racing off with it. There is no undertaking more important than that of selecting the researchers of the next generation and, as everyone knows, these do not exactly coincide with the perfect examinees or the young politicians who most easily get academic recognition and research grants.

We cannot afford to let good researchers "just happen," losing an equal number in the wayside entanglements of our haphazard or unimaginative promotion systems; we have to develop insightful systems for going out and finding them--for knowing where to look for gold before we actually see it glitter. And when we have found them we need to know something about the intellectual and emotional atmosphere in which they best grow—or, if you like, are "trained"—and the conditions of work that are most favorable for productivity.

If necessary, I could justify this call to "research on research" by an audit of the actual waste that now occurs, or by showing, in terms of the hundred thousand researchers now employed in government and industry, the millions spent on them and the still vaster millions they produce, that research is a major national concern. But, in the last resort, the importance of investigating research lies beyond these material considerations, in the desire for knowledge itself. In so far as we ardently desire that human understanding shall increase, we are concerned to sharpen the instruments of search, material and human; and in so far as we believe that the researcher's is a good life (approaching so closely to Plato's definition of ultimate happiness in a divine contemplation of the ordered working of the universe) we would wish that more could follow it, and with better opportunities for self-fulfillment.

Clearly, the problem of the researcher's personality and motivation requires an investigator with two qualifications: a sufficiently long and

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varied personal experience of research; and the special training of a psychologist in assessing personality and evaluating mental processes. To these I can make a moderate claim, with some slight added advantage of sympathy for the special viewpoints of brother scientists outside my area, engendered by being a renegade and sometime nostalgic chemist. Actually, the problem has been one of continual interest to me over thirty years, but whenever I was on the threshold of peeping into its secrets experimentally, the "Tally-ho" of my associates would call me back to the imperative scientific quarry of the day.

My chief hunting field during these years has been that of human personality and motivation, and, as scientists in other fields will recognize by analogy, the initial problem has been that of developing exact measuring and magnifying instruments, through observations by means of which, a reliable foundation could be formed for recognizing lawful relations and emergent concepts. However keenly a researcher on research may have wished to investigate the scientist's personality, he would have been compelled to recognize that, until an extremely short time ago, we did not possess the instruments and the theory to do so. In 1945, the available instruments could scarcely distinguish reliably between a librarian and a fighter pilot. Psychological journals are strewn with useless, premature attempts to apply personality measures to this or that selection problem—and, surely, the selection of such as the scientific researcher and the creative artist will demand the most subtle instruments in the laboratory. It has been the recognition of this fact which both helped to restrain my earlier interest in the problem and commanded my instant attention to it when the instruments became available. The instrument is still new and crude in many ways, and there are psychologists who will still question some of the concepts used; but the definiteness of the findings, and their presentation of an immediately useful practical selection formula, would seem to indicate that we have not struck before the iron was sufficiently hot to take useful shape.

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THE NEW YORK ACADEMY OF SCIENCES

announces

THREE PRIZE CONTESTS FOR 1955

The New York Academy of Sciences announces the following prize contests for the coming year:

I. THE A. CRESSY MORRISON PRIZES IN NATURAL SCIENCE

Two prizes of $300 each, to be known as the A. Cressy Morrison Prizes in Natural Science, will be awarded at the Annual Meeting of The New York Academy of Sciences in December 1955 for the two most acceptable papers in a field of science covered by the Academy or an Affiliated Society.

II. THE BORIS PREGEL PRIZE IN THE FIELD OF NATURAL RADIOACTIVE SUBSTANCES

Through the generosity of Doctor Boris Pregel, a prize of $500 is offered for the most acceptable paper embodying the results of research in the field of natural radioactive substances received in competition during the year 1955. The award of this prize will be announced at the Annual Meeting of the Academy in December 1955.

III. THE D. B. STEINMAN PRIZE FOR RESEARCH IN STRUCTURAL ENGINEERING

Through the generosity of Doctor D. B. Steinman, a prize of $500 is offered for the most acceptable original paper, submitted from the membership in the Academy, in the field of pure or applied research in structural engineering or the related science of aerodynamics or as applied to the analysis and design of civil engineering structures. The purpose is to encourage research development and writing in the subject engineering sciences. The award of this prize will be announced at the Annual Meeting of the Academy to be held in December 1955.

CONDITIONS GOVERNING THESE PRIZES

(1) *Eligibility*. Authors and co-authors competing for prizes I, and III shall be members in good standing of The New York Academy of Sciences or one of its Affiliated Societies, but nonmembers may become eligible by joining one of these organizations before the closing date.

Competition for prize II is open to all scientists engaging in research in the field of natural radioactive substances.

(2) *Date*. Papers are to be submitted on or prior to October 15, 1955, to the Executive Director of The New York Academy of Sciences, 2 East Sixty-third Street, New York 21, New York.

(3) *Papers*. All papers submitted must embody the results of original research not previously published. The manuscript shall be typewritten, in English, accompanied by all necessary photographs, drawings, diagrams, and tables, and shall be ready for publication. Papers must be accompanied by a summary of the data presented and conclusions reached.

(4) *Awards*. The awards shall be made by the Council of The New York Academy of Sciences. The decision of the judges shall be final.

(5) *Publication*. The Academy shall have first option on the publication of all papers submitted, unless especially arranged for beforehand with the authors, but such publication is not binding on the Academy.

(6) Wherever and whenever published, the papers awarded the prizes shall be accompanied by one of the three following statements:

In competition I, "Awarded an A. Cressy Morrison Prize in Natural Science in 1955 by The New York Academy of Sciences";

In competition II, "Awarded a Boris Pregel Prize for Research on Natural Radioactive Substances in 1955 by The New York Academy of Sciences";

In competition III, "Awarded a D. B. Steinman Prize for Research in Structural

Engineering in 1955 by The New York Academy of Sciences."

Such a statement, in substance, must also accompany any formal publicity initiated by the author regarding the prize paper. If published elsewhere with the consent of The New York Academy of Sciences, six copies of each prize paper must be deposited shortly after publication with the office of The New York Academy of Sciences.

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Eunice Thomas Miner
Executive Director

SECTION OF GEOLOGY AND MINERALOGY

GIANT DINOSAURS*

By Edwin H. Colbert

Curator of Fossil Reptiles and Amphibians, American Museum of Natural History, and Professor of Vertebrate Paleontology, Columbia University, New York, N. Y.

Almost all educated men know that there were dinosaurs on the earth in the far distant past, but not so many people know just what the dinosaurs were. Perhaps the most prevalent ideas about dinosaurs are that they were all gigantic animals (which is not strictly true); that, because of their great size, they became extinct many millions of years ago (which is almost surely not the reason for their extinction); and that they were "cold-blooded" reptiles (which is true).

Two orders of ancient reptiles, known as the Saurischia and the Ornithischia, contain the animals that we call dinosaurs. These two orders were related to the thecodonts, another reptilian group long since extinct; to the pterosaurs or flying reptiles, also extinct; and to the crocodilians, which are still with us. All of these reptiles had a common ancestry that dates back to the end of Paleozoic times, about 200 million years ago. The first dinosaurs appeared during the Triassic period of the Mesozoic era. From then on through the Jurassic period, and until the end of the Cretaceous period, the dinosaurs were destined to rule the earth's land masses. These reptiles were spectacularly dominant for a span of perhaps a hundred million years or more, but at the end of the Cretaceous period, they suffered complete extinction, as did various other groups of Mesozoic reptiles.

The dinosaurs were not all giants. Some of these ancient reptiles were no larger than turkeys or ostriches. Nevertheless, it is a fact that giantism was typical of their evolutionary history; so that of about 250 described genera of dinosaurs now known, at least 150 were giants. The fossil skeletons of some of these tremendous and impressive reptiles loom large in the halls of various museums, and create the general impression that all the dinosaurs were of great size.

What do we mean when we speak of the prevalence of giants among the dinosaurs? We are apt to think of ourselves as being of the "right size," so we regard as giants all those animals considerably larger than ourselves. If we were the size of squirrels we would live in a world largely inhabited by giants. If we were the size of elephants we would live in a world populated for the most part by pygmies. Giantism is a relative term. For the purposes of the present discussion, let us define giants as animals having a minimum length of 20 feet and a probable minimum weight in life of two tons. In human terms, animals of this size are incontestably giants.

*This paper, illustrated with lantern slides, was presented at a meeting of the Section on December 6, 1954.

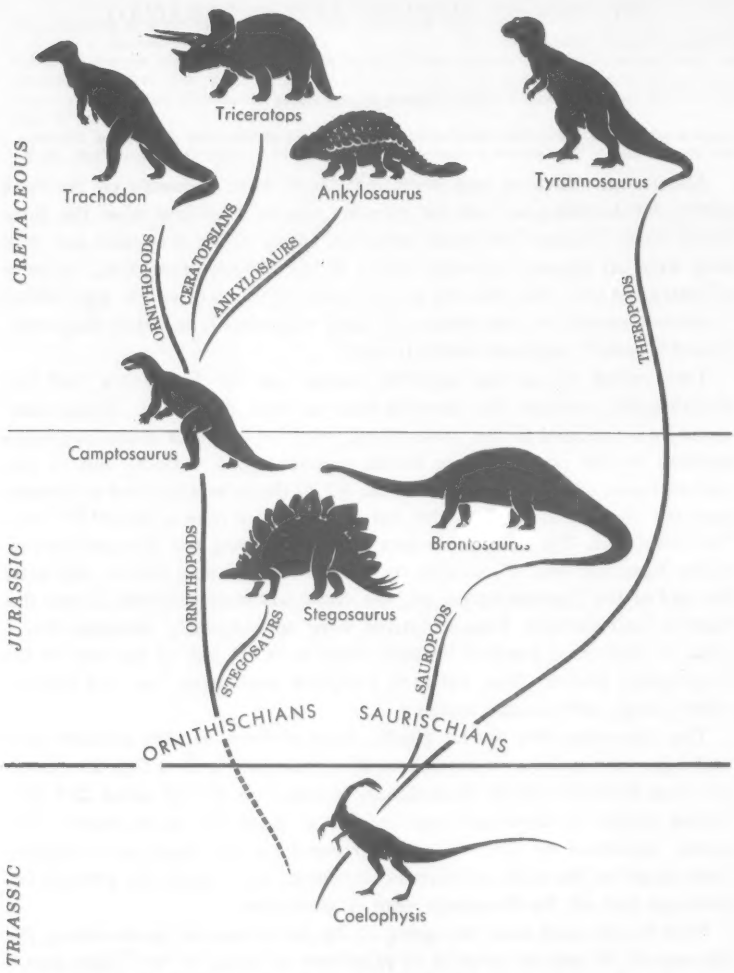

FIGURE 1. The evolution and relationships of the two orders and six suborders of dinosaurs. From E. H. Colbert, "Evolution of the Vertebrates," published by John Wiley and Sons, Inc., New York, 1955.

According to the terms of our definition, the early dinosaurs of Triassic days were rarely giants. With the progression of time through the Jurassic and Cretaceous periods, many of these reptiles evolved into giants, so that, among the later dinosaurs, the giants outnumbered the medium-sized and small forms by a wide margin. Middle and late Mesozoic times truly constituted an age of giants.

Why were so many of the dinosaurs giants? Before attempting to answer this question, let us examine some of the problems of giantism. Let us see, if we can, what are the advantages of being a giant and, conversely, what are the disadvantages.

The business of growing up to be a giant is not as simple in nature as it is in the fairy tales. The simple physical relationships of mass to linear dimensions and to bone strength make for definite limitations that restrict giants, particularly those that live on the land. As an animal increases in length, its mass increases roughly by the cube of the linear increase, while the strength of the supporting bones increases by the square of the linear increase. Therefore, when an animal gets progressively larger, its weight increases at a much faster rate than does the strength of its internal supports. Consequently, a point is finally reached where the animal cannot get any larger, because bones, ligaments, and muscles will not support its weight. It is very probable that the largest dinosaurs represent the top limits of size that a land-living animal can attain, and that the largest modern whales represent the greatest size attainable among water-living animals.

The problems of large size are not only those of physics, but are also problems of physiology and environment. For instance, a large animal requires a large area for its support. We know from the fossil evidence that extinct elephants, once inhabiting certain islands in the Mediterranean and off the California coast, were much smaller than their mainland relatives. The island areas were not extensive enough to support large mammoths. Again, we know from the study of modern animals that tigers in the East Indies do not occur on small islands. They require more area for their support than a small island affords.

Another problem that confronts the giant is one of protection from the elements. A small or medium-sized animal can get under cover when the weather is cold, or rainy, or when the sun is very hot. A giant cannot do this, but must stay out in the open and suffer. This problem of shelter was particularly important for reptilian giants such as the dinosaurs, whose temperature was directly proportional to the temperature of the environment.

The adverse physical effects of extremely large size, the need for large food-gathering areas, and the exposure to the elements that a giant must undergo, are some of the disadvantages of giantism. What, on the other hand, are some of the advantages?

As for physical relationships, the giant is fortunate in having a relatively small area of body surface in relation to mass. This factor is especially important for a reptile, which has no internal mechanism for maintaining body temperature. A large crocodile or alligator heats up slowly when exposed to the sun, and it cools off slowly when placed in the shade. Thus its body temperature fluctuates less than does the temperature of a small reptile, such as a lizard. Truly gigantic reptiles, such as the

dinosaurs, must have had fairly constant body temperatures, and this factor might have given them some of the advantages that are generally enjoyed by the so-called "warm-blooded" animals.

Because of the relationship of surface area to mass, the giant is more economical of energy than is the small animal. Consequently, the giant eats much less food in relation to its size than the small animal. For instance, among the mammals a shrew may eat as much as double its own weight of food every day. It has to do so to stay alive, for the great amount of surface area in relation to weight in this tiny animal causes a rapid loss of heat and of energy. An elephant, on the other hand, does very well on a small fraction of its own weight in daily food intake. This factor is also of great importance to reptilian giants.

Although the giant is exposed to the elements, he is generally safe from other animals, except other giants. His size is his protection. A full-grown elephant on the African veldt need fear no animal except a gun-toting man. The giant reptiles of Mesozoic times had only other giant reptiles as enemies.

It is apparent, then, that there are various advantages in being a giant, especially a reptilian giant. And it would seem that, during the Mesozoic era, the advantages outweighed the disadvantages in the evolutionary history of the dinosaurs.

Most of the giant dinosaurs were plant-eating reptiles. This fact is one indication as to the abundance of plant food that was available to those reptiles during middle and late Mesozoic times. Being reptiles of such size that they were not able to retreat underground for protection against adverse temperatures, it seems obvious that the giant dinosaurs must have lived in tropical and subtropical environments, where there was generally an abundance of lush vegetation. With large amounts of plant food available, conditions were favorable for the rapid evolution and continued development of these reptilian giants.

The wide distribution of giant dinosaurs over all the continents during the Mesozoic era is one line of evidence that tropical and subtropical environments were general throughout the world in those ancient days. Dinosaurs are found from Canada almost to the tip of South America, and from northern Europe and Mongolia to the southern tip of Africa. Therefore, it is reasonable to assume that much of the world was warm when the dinosaurs were living, and that climates like the present climate of southern Florida prevailed in what are now regions of hot summers and severely cold winters.

Although most of the giant dinosaurs were plant-eating reptiles, the first dinosaurs of late Triassic age were predominantly carnivores that preyed upon other contemporaneous reptiles. These were the early saurischian theropods; small to medium-sized dinosaurs that walked in a semi-erect posture on strong, birdlike hind legs; using the front feet like hands to assist in gathering food; and possessing light, strong skulls and

jaws furnished with sharp, bladelike teeth. Small, lightly-built carnivores such as these were the ancestors of the giant Jurassic and Cretaceous meat-eaters that preyed upon the huge plant-eating dinosaurs of those times. It is interesting to see the various adaptations that took place among the carnivorous dinosaurs during their growth to middle and

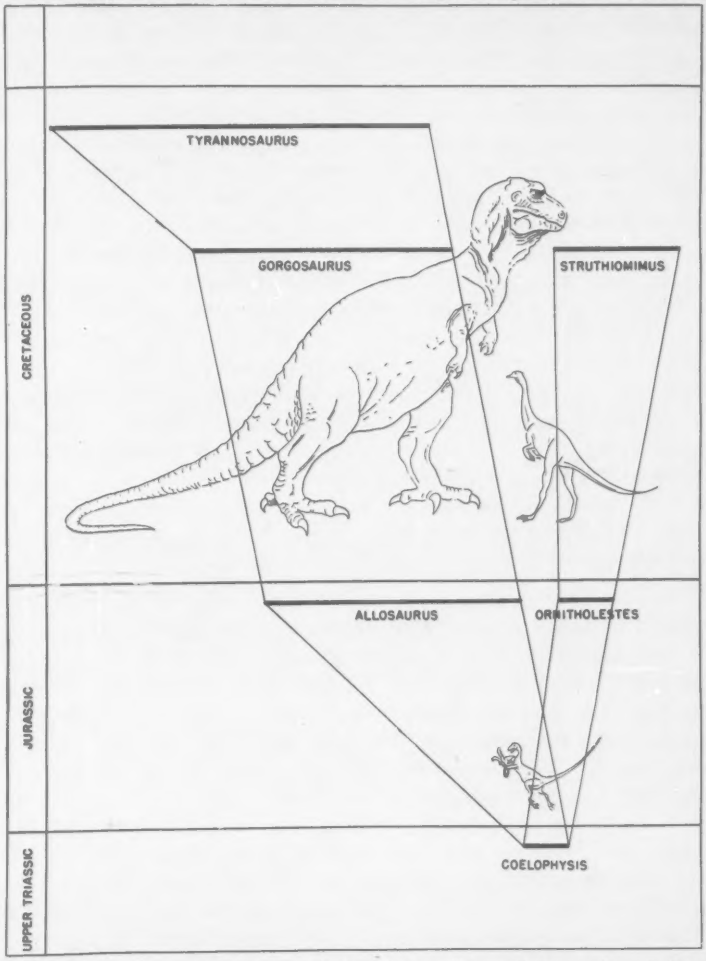

FIGURE 2. Size increase through time in two lines of theropod evolution. The black lines show comparisons of total lengths, all drawn to the same scale. The three restorations, drawn to scale, give an indication of the increase in mass of the culminating forms, *Tyrannosaurus* (left) and *Struthiomimus* (right), from an ancestral type (here represented by a drawing of *Ornitholestes*, a Jurassic theropod that was similar in size to *Coelophysis* and other Triassic dinosaurs.) From Colbert, 1949.

late Mesozoic giants. The leg bones, which had been hollow, became solid and heavy. The hind feet, which had been narrow and of graceful construction, became broad to support the great weight of the body, and the metatarsals (the upper long bones of the feet), became closely appressed to form a solid structure. The skull was inordinately enlarged, and this increase in size allowed a very wide gape to the toothed jaws—a most useful adaptation for a gigantic animal that preyed upon other giants. The extreme enlargement of the skull in the late Mesozoic meat-eating dinosaurs would have been impossible had it not been for accompanying adaptations that lightened the skull, making of it an open structure of bony bars that afforded bases for strong muscle attachments, yet, at the same time, cut down the "dead weight" of bone where it was not needed. Even so, the skull in these predators was heavy and considerable weight was added by the powerful jaw muscles. Accordingly, the neck was short, to make a short but strong lever between the head and the body. Since most of the functions of predation seem to have been concentrated in the great jaws of these dinosaurs, the front limbs which, in the ancestral forms, had been like arms and hands to assist in the capture of food, were reduced to ridiculously small appendages.

We might say that, among the theropod dinosaurs, the adaptations of the progenitors were magnified so that the giant descendants were able to pursue and kill their giant cousins. And we might further say that the evolution of these carnivores was dependent upon the rise and development of varied plant-eating forms which, in turn, evolved in close correlation with the spread of middle and late Mesozoic tropical and subtropical jungles.

The sauropods were the saurischian dinosaurs that evolved in the direction of an herbivorous diet. These were the gigantic brontosaurs, the largest of all dinosaurs, the reptiles that typify to most people the great rulers of the Mesozoic world. The brontosaurs attained such immense size that they were of necessity quadrupedal, thus departing from the bipedalism of their ancestors. The legs were very heavy and the feet were broad. The body was massive. The neck and tail were long, and the head was comparatively small. The backbone in these dinosaurs was like a bridge truss between the heavy piers of the legs. It was composed of large vertebrae, strongly interlocked by rather complicated articulations and lightened by the development of open spaces or cavities to cut down "dead weight." The jaws were furnished with rather weak rodlike or spoonlike teeth, capable of cropping soft vegetation, but nothing more. It seems evident that the sauropods, which must have commonly weighed 30 or 40 or 50 tons in life, were semiaquatic herbivores that usually waded in swamps, in rivers, or in lakes, where their great weight could be partially buoyed up by the water and where they might seek safety from the attacks of the giant predatory dinosaurs. These adaptations were highly

successful, for the brontosaurs continued until near the end of Cretaceous times and were widespread throughout the world.

The saurischian dinosaurs, which have so far been described, were the meat-eaters and the giant brontosaurs. The ornithischian dinosaurs, seemingly arising at a later date than the saurischians, were all plant-eating animals. They were much more varied than the saurischian dinosaurs, evolving in four distinct lines during Jurassic and Cretaceous times. Among the ornithischians, the ornithopods began as small to medium-sized, partially bipedal dinosaurs, known as camptosaurs. The camptosaurs had low skulls and, in front, their jaws formed a sort of flat beak that must have been useful for cropping vegetation. On the sides of the jaws were small, cutting teeth for chopping up green stuff. From the camptosaurs, the later ornithopods evolved and, of these, the most numerous were the hadrosaurs or duckbills, so named because the front of the skull and jaws formed a great flat beak, like that of an outsized duck. These were gigantic water-loving dinosaurs that waded around in shallow water to feed and that probably swam away into deep water when they were attacked or threatened by the big carnivorous dinosaurs. They showed many of the adaptations for giantism that we have already seen: powerful, heavy hind limbs and broad feet, and a strengthened backbone (frequently braced in these dinosaurs by a lattice work of ossified tendons).

The ornithischian stegosaurs were, essentially, enlarged camptosaurs that had become so huge that they went about on all four legs. Their legs were heavy and rather straight. The feet were short and broad, something like the feet of elephants, clearly an adaptation for bearing weight. The curved back of the stegosaurian dinosaurs was decorated by a double row of huge triangular plates that ran from the head down to near the end of the tail. On the tail were long spikes, which these animals could use to spear their enemies at close range by whipping their tails around. Of all the larger categories of dinosaurs, the stegosaurs alone failed to live to the end of Cretaceous times.

The ankylosaurs were the armored ornithischians. They were stegosaur-like in the general build of the skeleton, but were broader in body. The top of the head and the entire upper surface and sides of the body were encased in a solid mosiac of heavy bony plates. The end of the tail was armed with a huge mass of bone forming a club, or sometimes armed with spikes. Again, we see adaptations for the support of weight in these giants, particularly in the strong limbs and the very broad feet.

The last of the ornithischians to evolve were the ceratopsians or horned dinosaurs, which probably did not appear until late Cretaceous times. These dinosaurs had a very rapid evolutionary history, developing from 6-foot long ancestors to 25-foot long descendants within the span of a few million years. They were quadrupedal types with solid bodies, strong legs, and broad feet. The head in these dinosaurs was enormous, generally constituting about a third or a fourth of the total length. In front it was

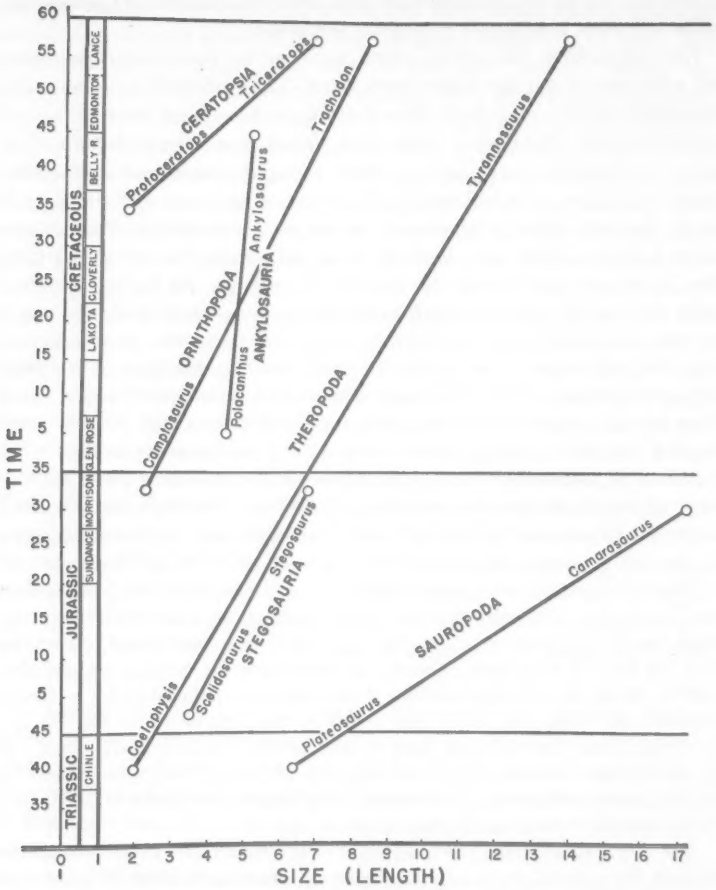

FIGURE 3. Increase in total length through time in the six suborders of of dinosaurs. For each suborder a straight line connects an early, primitive genus and a late, specialized genus. The slope of the line gives some indication of the evolutionary growth rate in each suborder. From Colbert, 1948.

narrow, like the beak of a parrot; in the back it flared to form a great frill that extended back over the shoulders. Above the eyes and on the nose there were commonly horns; in some, a long nasal horn, in others, long brow horns. The large head, with its horns, formed a powerful combination of spear and buckler, with which these plant-eating dinosaurs could fight their enemies, the giant predatory dinosaurs. The large frill that made up the back portion of the skull in the horned dinosaurs was, to a considerable degree, an enlarged origin for strong jaw muscles, but

it also served for the attachment of heavy neck muscles that gave great force to the thrust of the horns.

Except for the duck-billed dinosaurs, the ornithischians were predominantly "upland" dinosaurs that wandered around on dry land in search of a living. Therefore, they never reached the extremes of giantism that are seen in the swamp-living brontosaurs or even in the large hadrosaurs. They were preponderantly giants, nevertheless, and developed the varied adaptations that go along with giantism.

So it was that, at the end of Cretaceous times, a varied concourse of of dinosaur giants inhabited the continents of the earth. There were huge brontosaurs, great duck-bills or hadrosaurs, heavy ankylosaurs or armored dinosaurs, and big, rhinoceroslike ceratopsians, all feeding upon the abundant vegetation of a tropical and subtropical world; and always in the offing were the giant meat-eaters, lurking in readiness to attack the plant-eaters. Then, these numerous and seemingly successful dinosaurs of late Cretaceous times were, in a geological sense, quickly and completely exterminated during the transition from Cretaceous to Tertiary times. What were the reasons for the extinction of the dinosaurs?

It has frequently been said that the dinosaurs became extinct because they were "too big." They have been held up as fine examples of unsuccessful giants, of animals that grew to such huge dimensions they were unable to continue their struggle for existence. This easy expla-

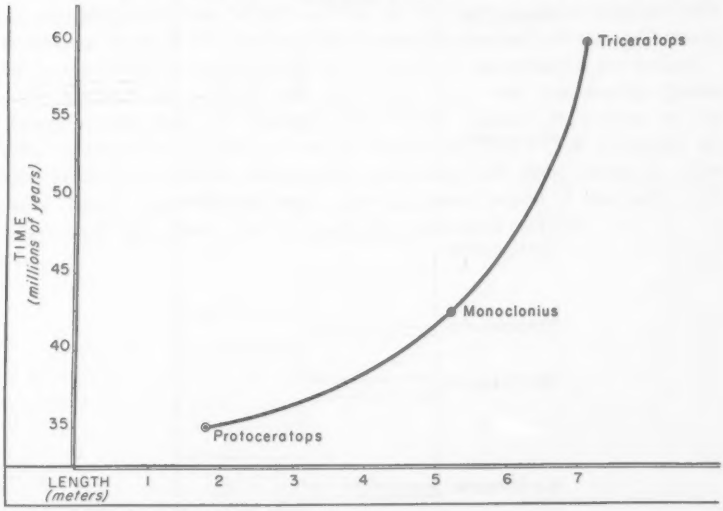

FIGURE 4. Increase in total length through time in the horned dinosaurs. This chart shows that most of the growth to giantism took place during the early phase of evolution, from *Protoceratops* to *Monoclonius*. Size increase from *Monoclonius* to *Triceratops* was comparatively slow. From Colbert, 1948.

nation of the disappearance of the dinosaurs is to be found in many a sober textbook.

It makes a nice story for moralists (Beware of the sins of bigness!), but is simply will not stand up under close scrutiny. Were the dinosaurs unsuccessful animals? They had an evolutionary history that ran for more than a hundred million years, which is a pretty good record, any way you look at it. Did they become extinct because they were giants? They were giants for many millions of years before their extinction, and there is no indication whatsoever that giantism was detrimental to their continued evolution. Indeed, the largest of the dinosaurs, the sauropods, had a particularly long evolutionary history, and they were seemingly prospering in late Cretaceous times, along with the various ornithischian dinosaurs so characteristic of that phase of geologic history.

No, the extinction of the dinosaurs cannot be attributed to giantism, for it is a fact that all of the dinosaurs died out at the end of the Cretaceous period, not just the giants. Why, for instance, did not some of the

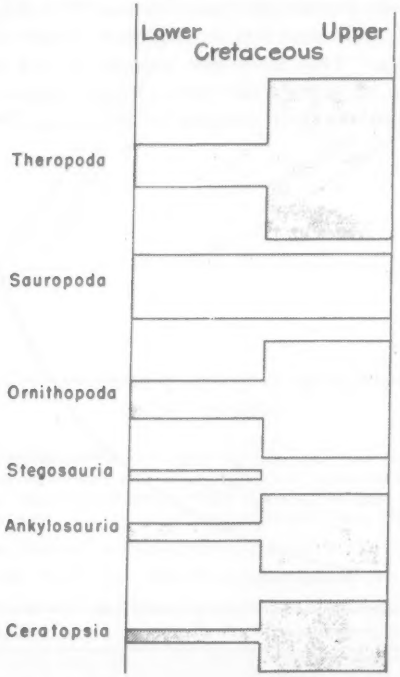

FIGURE 5. Extinction of the dinosaurs at the end of the Cretaceous period. The widths of the shaded areas are in proportion to known genera. Modified from Colbert, 1949.

small dinosaurs of late Cretaceous times live on into the Tertiary period, along with their crocodilian relatives?

Theoretically, the large size of the late Cretaceous dinosaurs might have been a factor for survival rather than for extinction. It was pointed out in a previous paragraph of this paper that, because of their great size, which would have caused slow, rather than rapid fluctuations of the body temperature, the giant dinosaurs might have had some of the attributes of "warm-blooded" animals. One might expect reptiles so favored to have lived on in a changing world. Yet all of the dinosaurs became extinct.

Extinction is one of the most difficult of all evolutionary phenomena to understand. Why was the whole series of extinctions that marked the end of the Cretaceous period along such strict taxonomic lines? Why did it affect not only the two orders of dinosaurs, but the flying reptiles as well, and, in addition, the dominant marine reptiles that flourished in the Cretaceous seas? Why did all of these animals disappear with such geological suddenness, when almost to the time of their extinction they were seemingly successful and flourishing organisms?

These are only a few of the many questions that can be asked in connection with the extinction of reptiles at the close of Mesozoic times. Extinction is obviously a complex phenomenon and, at times, a very subtle one. No single explanation is going to tell us why the dinosaurs and other reptiles vanished from the earth, and even a theory that involves multiple factors fails to satisfy us on many points connected with the extinction of these reptiles. It is indeed probable that we shall never reach a satisfactory solution to the problem of dinosaurian extinction.

Of this we can be sure: the dinosaurs were very successful giants through a long span of geologic time. Their sojourn as giants on the earth continued for as long a time as conditions favored the existence of such land-living giants. When they died, they left their bones in the earth. Today, one mammal, man, digs up these relics of the past and learns much from them and, at times, is confounded by them.

SECTION OF PHYSICS AND CHEMISTRY

CHEMOTHERAPY OF HUMAN CANCER*

By Jane C. Wright

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The treatment of cancer in humans presents one of the most challenging problems in medicine today. Among men and women of all ages, cancer ranks second in the causes of death in the United States while, in women between the ages of 35 and 54, it is first as a cause of death. In the vital statistics of the United States for the year 1953, there were 1,518,600 deaths from all causes, 566,420 deaths recorded due to heart disease, and 229,110 recorded due to cancer.

It has been estimated that only 30 per cent of patients with neoplastic diseases are alive today after five years by the use of the currently available methods of treatment by surgery and radiation therapy. Because of the limitations of surgery and radiation therapy in effecting a cure for cancer, chemotherapy is being explored with the hope of finding some agent which will cure neoplasms without harming the normal systems, functions, or cells within the body. Although a chemotherapeutic cure for cancer remains to be discovered, some satisfaction may be found in the progress which has been made in this direction in the last 12 years. While a number of chemotherapeutic agents are of value in the inhibition of animal tumors, they may not be of value in man. Hence the ultimate test of these agents lies in the evaluation of their effects in human cancer.

In this paper I shall briefly discuss the chemotherapeutic agents which are of some value today in the control of disseminated neoplastic disease in humans, and present our experiences with them.

TABLE 1 shows the chemotherapeutic agents which are presently of value: (1) the folic acid antagonists: Aminopterin, A-Methopterin, A-Ninopterin, Adenopterin, and Amino-an-fol; (2) the purine antagonist (6-mercaptopurine); (3) the nitrogen mustards: the aliphatic compound, methyl-bis(β-chlorethyl)amine hydrochloride and the aromatic, CB-1384; (4) the ethyleneimines: triethylene melamine, triethylene phosphoramide, and triethylene thiophosphoramide; (5) Myleran, or GT-41; (6) urethane; (7) Fowler's solution; and (8) the hormones: the adrenocorticotropic hormone, cortisone, estrogen, and testosterone.

*This paper, illustrated with lantern slides, was the first of two papers presented at a meeting of the Section on December 7, 1954.

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TABLE 1

CHEMOTHERAPEUTIC AGENTS OF SOME VALUE IN THE TREATMENT OF DISSEMINATED NEOPLASTIC DISEASES IN HUMANS

(1) Folic acid antagonists
- Aminopterin
- A-Methopterin
- A-Ninopterin
- Adenopterin
- Amino-an-fol

(2) Purine antagonists
- 6-Mercaptopurine

(3) Nitrogen mustards
- Aliphatic—HN_2
- Aromatic—CB—1348

(4) Ethyleneimines
- Triethylene melamine
- Triethylene phosphoramide
- Triethylene thiophosphoramide

(5) Myleran—GT 41

(6) Urethane

(7) Fowler's solution—potassium arsenite

(8) Hormones
- Adrenocorticotropic hormone
- Cortisone
- Estrogen
- Testosterone

Other chemotherapeutic agents which are of historical importance in the field of chemotherapy and/or some which have proven too toxic for practical clinical use, but do have some clinical effect on human tumors have been omitted, such as Coley's toxins, Shear's polysaccharides, alpha peltatin, *etc.* Other agents have been omitted either because insufficient clinical studies have been reported or because they are not, apparently, of significant clinical value.

The folic acid antagonists developed by the late Doctor Y. SubbaRow and his co-workers (1947) at the Lederle Laboratories, Pearl River, N. Y., comprise a group of substances which are related chemically and have similar biological actions.[1,2] Under the direction of Doctor James Williams, chemists of the Lederle Laboratories and the Calco Chemical Division of the American Cyanamid Company have synthesized and have been responsible for many of the chemical and biological developments of these agents. Basically, the folic acid antagonists are composed of a pteridine nucleus linked to a para-aminobenzoic acid structure as illustrated in FIGURE 1. Variations in the chemical structure of the different folic acid antagonists depend upon different substitutions at R_4, R_9', R_{10}'' and R'''.[3] TABLE 2 shows folic acid, substitutions in the basic formula of five of the antagonists and a derivative, Teropterin.[3] Biologically, the folic acid antagonists

TABLE 2

FOLIC ACID AND MODIFICATIONS OF THE BASIC FORMULA

Trivial name	*Generic name*	*R-4*	*R'9*	*R''10*	*R'''*
Folic acid	Pteroyl glutamic acid	OH	H	H	Glutamic acid
Teropterin	Pteroyl-y-glutamyl-y-glutamyl-glutamic acid	OH	H	H	-y-glutamyl-y-glutamyl glutamic acid
Aminopterin	4-aminopteroyl glutamic acid	NH_2	H	H	Glutamic acid
A-Methopterin	4-amino-N^{10}-methylpteroyl-glutamic acid	NH_2	H	CH_3	Glutamic acid
A-Ninopterin	4-amino-9-methylpteroyl-glutamic acid	NH_2	CH_3	H	Glutamic acid
Adenopterin	4-amino 9, N^{10}-dimethyl pteroyl glutamic acid	NH_2	CH_3	CH_3	Glutamic acid
Amino-an-fol	4-aminopteroyl aspartic acid	NH_2	H	H	Aspartic acid

oppose the action of folic acid *in vitro* and *in vivo*.[1,2,4,5,6] Folic acid and its biologically active form, the citrovorum factor,[7,8] are essential nutritional factors present in all tissues, but present in highest concentration in tissues in a state of rapid growth.[9,10] Diets deficient in folic acid produce effects in animals similar to those produced by the folic acid antagonists. The effects produced in animals with the antagonists have been wholly or partially reversible with folic acid, but completely reversible with the citrovorum factor.[11,12,13,14,7,15,16] Studies on the mechanism of action of the folic acid antagonists indicate that these substances interfere with nucleic acid synthesis.[14,17,18,19,20,21,22]

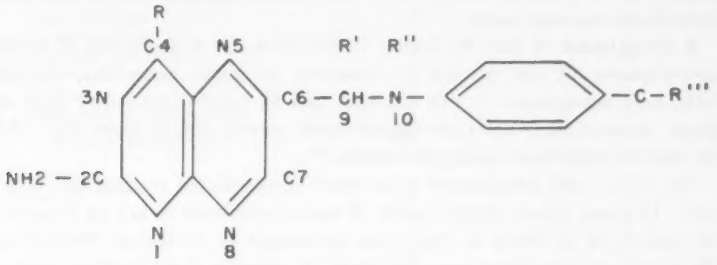


FIGURE 1

In 1948, Doctor Sidney Farber reported his epoch-making discovery that the folic acid antagonists produced remissions and an increase in the life span of children with acute leukemia.[23,24] Prior to this observation, there was no therapy, medical, surgical, or radiological, of any significant value for children with acute leukemia. In the prefolic acid antagonist era, children with acute leukemia rarely lived for one year after the diagnosis was established and the spontaneous remission rate varied from 1 to 10 per cent in these cases.

The folic acid antagonists of most clinical value are Aminopterin, A-Methopterin, A-Ninopterin, Adenopterin and Amino-an-fol. Today the most widely used are Aminopterin and A-Methopterin.

The usual daily oral dose employed is 0.25 to 1 mg. with Aminopterin, and 2.5 to 10 mg. with A-Methopterin. These drugs are given daily, by some, with discontinuance when toxicity occurs, and intermittently by others. No clear-cut relationship exists between the amount of the drug given and its clinical effect. Among adults, we have found that the tolerated daily dosages vary greatly among individuals.

The greatest value of the folic acid antagonists lies in the treatment of the acute leukemias in children. When children with acute leukemia respond to the action of these drugs, improvement is usually recognized two to three weeks after the onset of therapy, and consists of a reduction in the enlarged lymph nodes, liver, spleen, a decrease in or cessation of bleeding, a return to normal of an elevated temperature, a decrease

in bone pain, a return toward normal in the bone marrow and peripheral blood picture as manifested in the white blood cell count, red blood cell count, hemoglobin, platelet count, and percentage of blast cells associated with general clinical improvement. In a number of patients, both the clinical and hematological picture returns to normal.

Reports from numerous investigators at the Second Conference on Folic Acid Antagonists in Acute Leukemia held in Boston, Mass., on March 11, 1951, showed that the average remission rate in children with acute leukemia was about 50 per cent in those treated for one day or or longer with antifolic acid compounds.[25] Also reported were prolongations in the survival time. Some children treated with the antagonists have lived over two years.

A compilation of data by Doctor Farber from the experiences of seven investigators of the results of treatment of acute leukemia with the folic acid antagonists in 425 children and 52 adults treated 14 days or more, showed 68.5 per cent improvement among the children and 13.5 per cent improvement among the adults.[26]

The folic acid antagonists also exert some effects against solid tumors. In these cases the evidence of tumor inhibition is not as dramatic or marked as is found in the acute leukemias of childhood. Favorable objective changes have been reported in patients with lymphosarcoma, Hodgkin's disease, the chronic leukemias, neuroblastoma, rhabdomyosarcoma, carcinoma of the breast, and carcinoma of the bladder.[24,25,27-31]

At Harlem Hospital, we have treated 152 patients with incurable neoplastic diseases of various types with the folic acid antagonists. TABLE 3 shows the diagnosis of these cases, the number of each type and the number showing evidence of objective improvement. In this series of cases, the improvement generally consisted of temporary, slight to moderate reduction in the size of tumor masses. At times, this reduction was associated with general clinical improvement. Forty-nine cases of sarcomas and related diseases were treated. Twenty patients with sarcomas and related diseases showed objective evidence of improvement under this therapy. One hundred and three patients with disseminated carcinomatosis were treated with the folic acid antagonists; 23 of these patients showed some improvement.

It is in patients with carcinoma of the breast and of the bladder that most amelioration from antifolic therapy occurred among the carcinomatosis patients. When changes are produced in patients with carcinoma of the breast, they most frequently consist of reductions in the size of soft-tissue tumor masses. In two patients, healing of osteolytic bone metastasis occurred three and five months, respectively, after institution of therapy.

The toxic signs and symptoms which may result from the use of the folic acid analogs are mucous membrane ulcerations, nausea, vomiting,

TABLE 3

CLASSIFICATION AND RESULTS OF 152 CASES OF NEOPLASTIC DISEASES TREATED WITH FOLIC ACID ANTAGONISTS

Diagnosis	*Number treated*	*Number with objective improvement*
Sarcomas and related diseases		
Hodgkin's disease	7	5
Lymphosarcoma	5	3
Unclassified lymphomas	2	0
Chronic myelogenous leukemia	6	2
Chronic lymphatic leukemia	4	3
Acute reticulum cell leukemia	1	1
Acute myelogenous leukemia	7	3
Fibrosarcoma	3	1
Lymphoepithelioma	2	0
Multiple myeloma	8	1
Ewings bone tumor	1	0
Melanoma	2	0
Malignant neurilemmoma	1	0
Total (sarcomas)	49	20
Carcinomas:		
Breast	39	12
Prostate	8	3
Stomach	7	1
Liver	1	0
Pancreas	5	0
Cecum	2	1
Colon	2	0
Rectum	5	0
Kidney	1	0
Bladder	5	2
Lung	4	0
Cervix	1	0
Ovary	3	1
Skin	5	0
Glioblastoma	1	0
Metastic—1° site undetermined	14	3
Total (carcinomas)	103	23
Total for series	152	43

diarrhea, leukopenia, bleeding from the intestinal tract, and alopecia. Therapy should be immediately discontinued when signs of toxicity appear. The side effects then generally disappear within one week. If therapy is discontinued when stomatitis, one of the earliest side effects, occurs, hemorrhage from the intestinal tract will be prevented. The use of the citrovorum factor, leucovorin, in dosages of three mg. daily, intramuscularly, will accelerate the disappearance of the toxic effects produced by the antifolic compounds and, at the same time, will counteract the therapeutic action of the antagonist. No apparent demonstrable alteration in the growth or development of children has been observed by Farber in those treated for one year or more with these drugs.[26] In our adult patients, treatment has been continued in some cases for one,

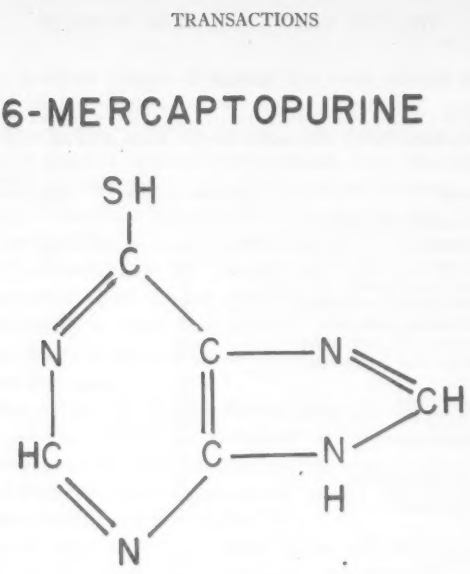


FIGURE 2

two, or three years with no apparent harmful effect. The production of toxicity with these compounds is not necessary for a therapeutic effect.[25,26]

Another antimetabolite which has been shown to be of clinical value in the therapy of acute leukemia is 6-mercaptopurine. Doctor George Hitchings and his associates at the Wellcome Research Laboratories at Tuckahoe, N.Y., developed and described the synthesis, chemical and biological properties of 6-mercaptopurine and related antagonists of nucleic acid derivatives.[32,33,34] The chemical configuration of 6-mercaptopurine is illustrated in FIGURE 2. 6-Mercaptopurine is a purine antagonist for the wild strain of the *Lactobacillus casei*. The inhibition in this system is prevented by any one of four purine bases, namely, adenine, guanine, xanthine, or hypoxanthine.[33,35] Tumor inhibition in mouse sarcoma 180 and in mouse leukemia occurs with the administration of 6-mercaptopurine.[36,37,38] In 1953, Doctor Joseph Burchenal and his associates demonstrated the antileukemic activity in man with 6-mercaptopurine.[38,39,40]

The average daily dose of 6-mercaptopurine orally is 2.5 mg. per kg. body weight. Generally three to six weeks of therapy are necessary to produce results with this analog.

In a series of 68 children with acute leukemia treated at Memorial Hospital with this purine analog, remissions were achieved in 29 patients, partial remissions in 14, and 25 were considered failures. Of

44 adults with acute leukemia, five had clinical and hematological remissions, six showed marked clinical benefit with some hematological improvement, and 29 were considered failures.[41] At a conference on 6-mercaptopurine April 30 and May 1, 1954, in New York City, clinical reports from a number of investigators confirmed the fact that 6-mercaptopurine was useful in the therapy of acute leukemia. Some patients in whom resistance develops from the use of A-Methopterin or cortisone may respond to the administration of 6-mercaptopurine.

We have treated 22 patients with neoplastic diseases at Harlem Hospital with 6-mercaptopurine. TABLE 4 shows the classification and results of these cases. Three patients with sarcomas and related diseases were treated with 6-mercaptopurine; one of these, a child with acute leukemia, experienced a remission. Nineteen patients with disseminated carcinomatosis were treated. In two of these cases, both with metastatic carcinoma of undetermined primary site, a definite but temporary reduction in the sizes of soft tumor masses occurred.

The most frequent side effect produced with the administration of 6-mercaptopurine is leukopenia. We have observed, also, gastrointestinal upsets in two patients and ulcerations at the angles of the mouth and in the mouth in two patients. Toxicological and pharmacological studies with 6-mercaptopurine in mice, rats, cats, and dogs showed disturbances referable to the hematopoetic system, gastrointestinal tract, and liver. In rats, myocarditis and pulmonary lesions also occurred.[37,42]

The next group of compounds of use in the treatment of the human neoplasms are the nitrogen mustards. Interest in this group of substances

TABLE 4

CLASSIFICATION AND RESULTS OF 22 CASES OF NEOPLASTIC DISEASES TREATED WITH 6–MERCAPTOPURINE

Diagnosis	*Number treated*	*Number with objective improvement*
Sarcomas:		
Hodgkin's disease	1	0
Lymphosarcoma	1	0
Acute leukemia	1	1
Carcinomas:		
Breast	3	0
Prostate	2	0
Stomach	1	0
Pancreas	1	0
Cecum	1	0
Colon	1	0
Rectum	2	0
Lung	1	0
Bladder	1	0
Ovary	1	0
Metastatic–1° site undetermined	5	2
Total	22	3

developed after pathological and biological studies were made following the use of dicloroethyl sulfide or mustard gas during World War I.

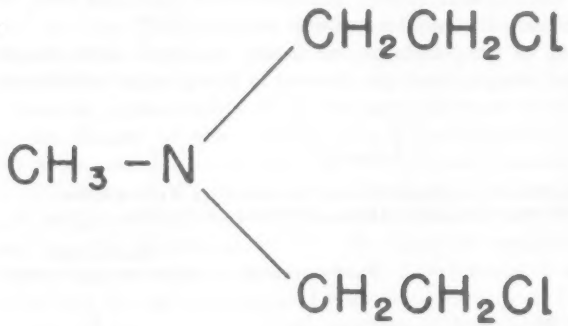
Gilman and Philips (1946) first observed improvement in human lymphosarcoma treated with the tris nitrogen mustard or tris (β-chorethyl)amine hydrochloride.[43] Subsequently, other investigators found the nitrogen mustards to be of value in the control of cases of similar neoplastic diseases.[44,45,46,47,48]

The chemistry and mechanism of action of the nitrogen mustards was reviewed by Ross in 1953.[49] Ross concludes that the outstanding feature of the group of cytotoxic agents, to which the mustards belong, is their ability to function as alkylating agents under the mild conditions arising in living tissues.

The most extensively clinically tested mustard is the aliphatic compound, methylbis(β-chloroethyl)amine hydrochloride referred to as HN_2. FIGURE 3 shows the formula of HN_2.

HN_2 is administered as a freshly made solution intravenously. The average dose per course is 0.1 mg. per kg. body weight daily for four days.

HN_2 has been found to be of temporary value in the control of cases of Hodgkin's disease, lymphosarcoma, the chronic leukemias, polycythemia



Methyl-bis(β-chloroethyl) amine hydrochloride (HN2)

FIGURE 3

rubra vera, and bronchogenic carcinoma. In these cases, the response to mustard therapy is manifested by reductions in tumor size and amelioration of symptoms produced by neoplastic infiltrations. The best results from the use of the mustards have been seen in cases of Hodgkin's disease, lymphosarcoma, chronic myelogenous leukemia, and polycythemia vera.

The use of the nitrogen mustards in the chemotherapy of human neoplastic diseases is restricted because of their marked clinical toxicity. Toxic effects of HN_2 are both local and systemic. The local effects occur at the site of injection. They are caused by the cytotoxic action of this agent and are characterized by a painful inflammatory reaction, thrombophlebitis, and localized necrosis. The systemic toxic effects are both immediate and delayed. The immediate changes are manifested by nausea and vomiting, which usually occur one to eight hours after administration of the drug and continue for 3 to 24 hours. The delayed effects of HN_2 are primarily due to damage of all elements of the hemopoetic system. Maculopapular skin eruptions may occur.

Recently another nitrogen mustard, an aromatic compound, CB-1348, from the Chester Beatty Research Institute in London, became available for clinical trial. Preliminary clinical trials with this compound indicate that it has some value in the therapy of the lymphomatous group of diseases.[50]

The next class of compounds of significant clinical value are the ethyleneimines. The ethyleneimines most frequently used clinically are triethylene melamine, triethylene phosphoramide, and triethylene thiophosphoramide. Triethylene melamine (FIGURE 4) is a drug which was first synthesized in Germany during World War II for use in a commercial process for improving the finish of rayon fabrics. In a screening program with agents capable of reacting with amino groups, such as urethane, and with reactive groups similar to the hydrolysis breakdown products of the nitrogen mustards, Rose and his associates found triethylene melamine to have outstanding growth-inhibitory activity against the Walker carcinoma 256.[51] In another screening program by Lewis and Crossley, triethylene melamine and related ethyleneimine derivatives were found to retard the growth of certain transplanted sarcomas and carcinomas.[52] Other studies showed triethylene melamine to prolong the survival time of mice with transmitted leukemia, and to inhibit the development of sarcoma 180 in mice.[53,54]

The structural configuration of triethylene melamine, triethylene phosphoramide, and triethylene thiophosphoramide are illustrated in FIGURES 4, 5, and 6.

The oncolytic effects produced by triethylene melamine, triethylene phosphoramide, and triethylene thiophosphoramide in human tumors have been reported.[55-66] The first of these reports was presented at a meeting

TRIETHYLENE MELAMINE

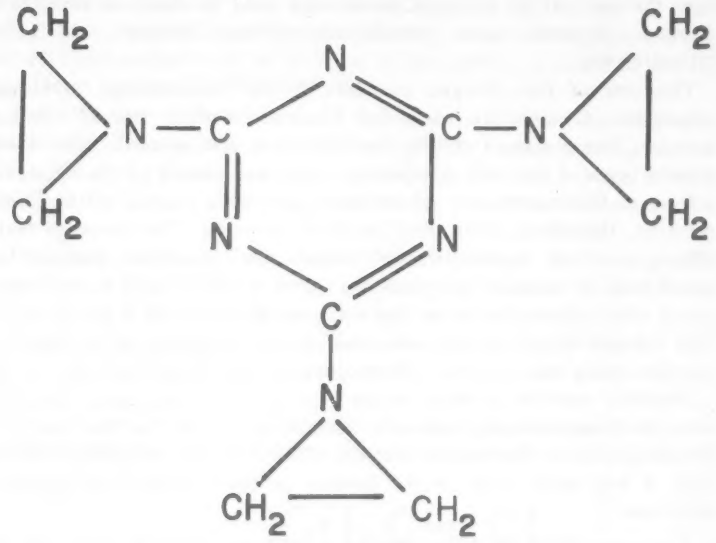


FIGURE 4

N,N',N''-TRIETHYLENEPHOSPHORAMIDE

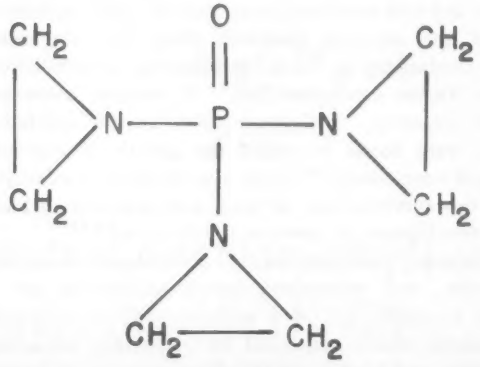


FIGURE 5

before The Association of American Physicians on May 2 and 3, 1950, in Atlantic City, N. J., by Doctor Cornelius P. Rhoads.[56]

At Harlem Hospital, we have had clinical experience with the use of triethylene melamine, triethylene phosphoramide, and triethylene thiophosphoramide in 122 patients with neoplastic diseases during the last four years.

The triethylene melamine, triethylene phosphoramide, and triethylene thiophosphoramide have been given both intermittently in courses and by maintenance therapy. The triethylene melamine was administered orally, and the triethylene phosphoramide and triethylene thiophosphoramide generally, intramuscularly, but also intrapleurally, intraperitoneally, and directly into the easily accessible soft tissue tumor masses. The average dose provided per course was 5 mg. of triethylene melamine daily, orally, for three days, or 5 to 10 mg. of triethylene phosphoramide daily, intramuscularly, for five to ten days, or 20 to 40 mg. intramuscularly, per week, of triethylene thiophosphoramide. Dosage schedules were constantly and frequently adjusted for each patient according to his or her hematopoetic sensitivity to the drugs. The hematopoetic systems of patients with chronic lymphatic leukemia and multiple myeloma were generally more sensitive to the drugs.

When improvement occurred following the administration of triethylene melamine, triethylene phosphoramide, or triethylene thiophosphoramide, it was first generally observed between the 3rd and 28th day succeeding initiation of therapy in those patients in whom remission was observed. It generally took between one to nine months to achieve a complete remission with an average time of three to six months. The smallest total dose which produced a complete remission was 15 mg. with triethylene melamine and 150 mg. with triethylene phosphoramide. The largest total dose required to produce a complete remission was 90 mg. with triethylene melamine and 175 mg. with triethylene phosphoramide. The duration of improvement displayed a high degree of variability, a

TRIETHYLENE THIOPHOSPHORAMIDE

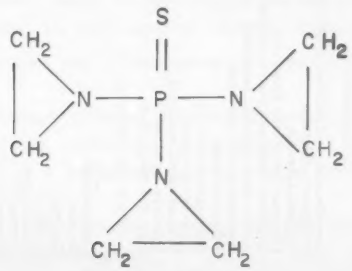


FIGURE 6

TABLE 5

CLASSIFICATION AND RESULTS OF 71 PATIENTS TREATED WITH TRIETHYLENE MELAMINE

Diagnosis	*Partial objective improvement*	*Complete remission*	*Total improved*	*Questionable improvement*	*Unimproved*	*Total number of cases*
I. *Lymphomas and related diseases:*						
Hodgkin's disease	7	3	10	–	1	11
Lymphosarcoma	2	4	8	–	1	7
Chronic lymphatic leukemia	4	–	4	–	–	4
Chronic myelogenous leukemia	1	1	2	–	–	2
Acute myelogenous leukemia	–	–	–	–	1	1
Reticulum cell sarcoma	3	–	3	–	–	3
Fibrosarcoma	5	–	5	–	–	5
Osteogenic sarcoma	–	–	–	–	3	3
Chondrosarcoma	–	–	–	–	1	1
Unclassified sarcoma	–	–	–	–	2	2
Multiple myeloma	2	–	2	1	5	8
Mycosis fungoides	1	–	1	–	–	1
Total (lymphomas)	25	8	33	1	14	48
II. *Carcinomas:*						
Tonsil	–	–	–	–	1	1
Larynx	–	–	–	1	–	1
Lung	–	–	–	–	4	4
Esophagus	–	–	–	–	2	2
Stomach	–	–	–	1	–	1
Large bowel	–	–	–	–	3	3
Kidney	–	–	–	–	2	2
Ovary	–	–	–	–	3	3
Breast	–	–	–	–	2	2
Skin	–	–	–	–	1	1
Metastatic—1° undetermined	–	–	–	–	3	3
Total (carcinomas)	0	0	0	2	21	23
Total lymphomas and related diseases	25	8	33	1	14	48
Total carcinomas	0	0	0	2	21	23
Total for series	25	8	33	3	35	71

characteristic already observed in the data on the amounts of the drug necessary to produce remission.

Of the 122 patients with incurable neoplastic diseases treated, 71 were treated with triethylene melamine, 39 with triethylene phosphoramide, and 12 with triethylene thiophosphoramide.

TABLE 5 shows the results of treatment of triethylene melamine in 71 cases. Of the 71 patients with incurable neoplastic diseases treated with triethylene melamine, 48 were diagnosed as lymphomas and related diseases, and 23 as carcinomas. Of the lymphoma group of patients, 25 patients showed partial evidence of objective improvement and, in 8, complete remissions were observed while the patients were under treatment with triethylene melamine. Complete remissions were obtained in three cases of Hodgkin's disease, four cases of lymphosarcoma, and one case of chronic myelogenous leukemia. There were partial evidences of objective improvement in Hodgkin's disease, lymphosarcoma, chronic lymphatic leukemia, chronic myelogenous leukemia, reticulum cell sarcoma, fibrosarcoma, multiple myeloma, and mycoses fungoides. The partial remissions lasted from 2 to 230 days. The complete remissions lasted from 56 days to 3 years. Repeat remissions were produceable.

Acute myelogenous leukemia, osteogenic sarcoma, chondrosarcoma, and unclassified sarcoma exhibited no favorable reaction to therapy. In the group of patients with carcinoma no definite clinical effect was observed with the use of triethylene melamine.

Of the 39 patients treated with triethylene phosphoramide, 25 were diagnosed as lymphomas and related diseases, and 14 as carcinomas. TABLE 6 shows the classification and results of treatment of the 39 patients. In the group of patients with lymphomas, partial objective evidence of improvement occurred in 11 cases, and complete remissions occurred in 3 cases. Complete remissions were obtained in one case of lymphosarcoma, one case of chronic myelogenous leukemia, and one case of reticulum cell sarcoma. There were partial remissions in Hodgkin's disease, lymphosarcoma, chronic lymphatic leukemia, reticulum cell sarcoma, fibrosarcoma, multiple myeloma, and malignant melanoma. The partial remissions lasted for 1 week to 120 days. The complete remissions lasted from 1 week, in the case of the reticulum cell sarcoma, to 203 days, to date, in one case of chronic myelogenous leukemia.

Osteogenic sarcoma and leiomyosarcoma exhibited no favorable reaction to therapy.

Among the carcinomas treated with triethylene phosphoramide, one patient with metastatic carcinoma of undetermined primary site, but probably lung, showed a reduction in tumor size under treatment with triethylene phosphoramide.

Carcinomas of the lung, stomach, cecum, rectum, breast, and prostate showed no significant improvement.

Twelve patients were treated with triethylene thiophosphoramide.

TABLE 6

CLASSIFICATION AND RESULTS OF 39 PATIENTS TREATED WITH TRIETHYLENE PHOSPHORAMIDE

Diagnosis	*Partial objective improvement*	*Complete remission*	*Total improved*	*Questionable improvement*	*Unimproved*	*Total number of cases*
I. *Lymphomas and related diseases:*						
Hodgkin's disease	3	–	3	1	1	5
Lymphosarcoma	3	1	4	–	1	5
Chronic lymphatic leukemia	1	–	1	–	–	1
Chronic myelogenous leukemia	–	1	1	–	–	1
Reticulum cell sarcoma	1	1	2	–	–	2
Fibrosarcoma	1	–	1	–	–	1
Osteogenic sarcoma	–	–	–	–	1	1
Leiomyosarcoma	–	–	–	1	–	1
Multiple myeloma	1	–	1	1	5	7
Malignant melanoma	1	–	1	–	–	1
Total (lymphomas)	11	3	14	3	8	25
II. *Carcinomas:*						
Lung	–	–	–	2	1	3
Stomach	–	–	–	–	3	3
Cecum	–	–	–	–	1	1
Rectum	–	–	–	1	–	1
Breast	–	–	–	–	2	2
Prostate	–	–	–	–	1	1
Metastatic–1° undetermined	1	–	1	1	1	3
Total (carcinomas)	1	0	1	4	9	14
Total lymphomas and related diseases	11	3	14	3	8	25
Total carcinomas	1	0	1	4	9	14
Total for series	12	3	15	7	17	39

TABLE 7 shows the results of treatment of these cases. Three patients showed some evidence of improvement.

With triethylene phosphoramide and triethylene thiophosphoramide therapy given intraperitoneally, some control of ascites has been observed in two patients, the one with a carcinoma of the ovary and the other with a carcinoma of the stomach.

PLATE 1 shows a roentgenogram of the chest of a patient with Hodgkin's disease taken before therapy with triethylene melamine. Observe the enlarged hilar nodes.

PLATE 2 shows an X ray of the same patient taken 106 days after onset of triethylene melamine therapy. PLATE 3 is an X ray of the same patient taken 13 months after onset of therapy. Observe the marked decrease which has taken place in the size of the hilar masses.

Rosenthal has reported the production of clinical and hematological remissions lasting on the average of 8 to 9 months in 20 out of 30 patients with polycythemia vera with the use of triethylene melamine.[67] Some control of breast and ovarian carcinoma occurs with the use of the triethylene thiophosphoramide, according to Shay and Bateman.[65,66]

The side effects from this group of compounds are not as devastating as those seen following HN_2 therapy. The main side effect is the production of leukopenia. Occasionally nausea, vomiting, and diarrhea are produced with large oral dosages of triethylene melamine. No G.I. symptoms are seen from triethylene phosphoramide or triethylene thiophosphoramide.

Myleran, or GT-41, was discovered and first studied by Haddow, Timmis, and Galton of the Chester Beatty Research Institute. It showed an intense inhibitory effect on the growth of the Walker rat carcinoma 256, did not depress lymphopoiesis appreciably at dosages which produced 50 per cent or more decrease in neutrophils, depressed the myeloid series in rats and man, and produced remissions or improvement in patients with chronic granulocytic leukemia.[67-77]

TABLE 7

CLASSIFICATION AND RESULTS OF 12 CASES OF NEOPLASTIC DISEASES TREATED WITH TRIETHYLENE THIOPHOSPHORAMIDE

Diagnosis	*Number treated*	*Number with objective improvement*
Sarcomas:		
Hodgkin's disease	2	0
Lymphosarcoma	1	0
Carcinomas:		
Breast	4	2
Stomach	1	0
Testicle	1	0
Metastatic—primary site unknown	3	1
Total	12	3

Clinical experiences with the use of Myleran by Petrakis *et al.* confirmed the previous observations and showed that this compound was an agent of some value in the therapy of chronic myelogenous leukemia in man.[78] FIGURE 7 shows the chemical structure of Myleran, or 1:4-dimethanesulfonyloxybutane.

Myleran is administered daily, orally, in dosages of 4 to 10 mg. for a total dose of approximately 200 to 500 mg. or in courses of 25 mg. for four to six days. It has a selective depressant effect on the granulocytic elements. Its most undesirable side action is the production of thrombocytopenia.

Urethane, a drug used for animal anesthesia, was discovered by

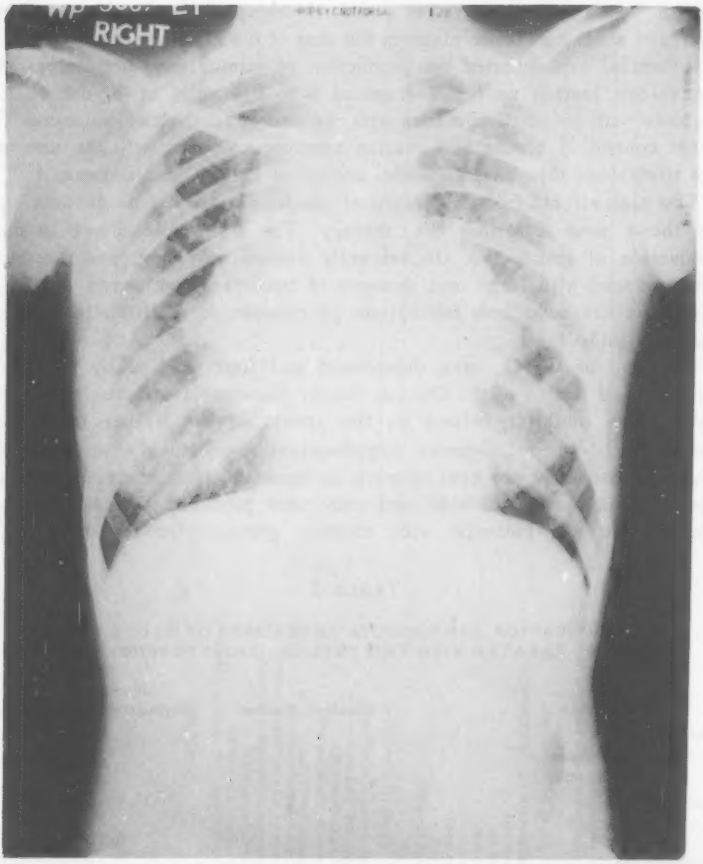


PLATE 1. A roentgenogram of the chest of a patient with Hodgkin's Disease taken before therapy with triethylene melamine. Observe the enlarged hilar nodes.

PLATE 2. An X ray of the same patient taken 106 days after onset of triethylene melamine therapy.

Haddow and Sexton to be active against animal tumors and, particularly, inhibitory against the Walker rat carcinoma.[79] FIGURE 8 shows the chemical configuration of urethane or ethyl carbamate.

Clinical investigations with urethane, by Paterson, Haddow, *et al.* showed urethane to have effects similar to radiation therapy in patients with leukemia.[80] In the most favorable cases, a fall in the total white blood cell count to normal, approachment of the differential count to normal, decrease in the size of the spleen and lymph nodes, and a rise in the hemoglobin occurs. These investigators reported, also, diminution in the size of lesions in 3 out of 13 patients with carcinoma of the breast, and in four with miscellaneous carcinomas. While urethane is

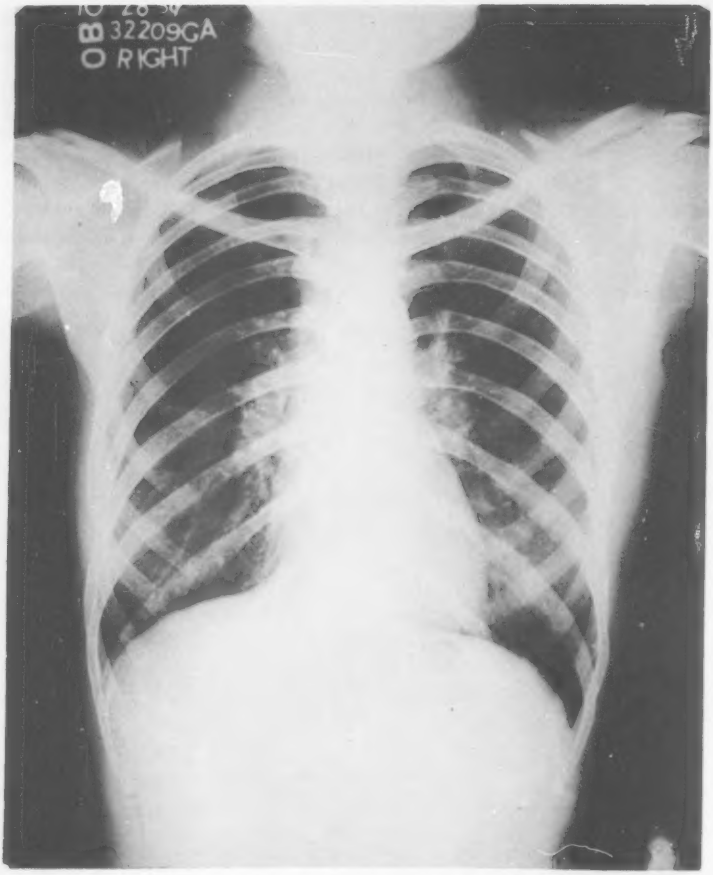


PLATE 3. An X ray of the same patient taken 13 months after onset of therapy.

effective in leukemia, especially the chronic myelogenous form, this agent, like other chemotherapeutic agents, has only a temporary antitumor effect.

In the therapy of multiple myeloma with urethane, dramatic results of improvement (as compared with the use of other agents in this disease) were reported by Loge and Rundles in 1949.[81] They described decrease in bone pain, decrease in fever, improvement in blood values, improvement in serum protein abnormalities, the albuminurea and Bence Jones proteinurea, decrease in abnormal plasma or myeloma cells, X-ray evidence of apparent cessation of progression of osteolytic bone lesions.[81-84]

MYLERAN

1-4-DIMETHANESULFONYLOXYBUTANE

$CH_3-SO_2-O-(CH_2)_4-O-SO_2-CH_3$

FIGURE 7

The oral administration of two to four gm. per day of urethane is the average dosage employed. Due to the development of nausea and vomiting from the administration of this drug, its usefulness as a chemotherapeutic agent in humans is limited.

In the later part of the 19th century, arsenic, in the form of potassium arsenite (Fowler's solution), was one of the most frequently employed of the diversified agents used in cancer chemotherapy. In 1865, Lissauer first described marked symptomatic improvement in a patient with leukemia following the administration of potassium arsenite or Fowler's solution.[85] The best effects with the use of this compound are seen in the therapy of chronic myelogenous leukemia; here remissions may be temporarily produced.[86,87]

Fowler's solution administration is initiated at five minims three times daily by mouth. The dosage is then gradually increased to tolerance level. As in the case of urethane, some patients develop nausea and vomiting, and hence are unable to tolerate the drug.

The last group of compounds, which have been shown to have temporary distinctive value in the therapy of disseminated neoplastic diseases in man, are the hormones. The hormones of value are the adrenocorticotropic hormone, cortisone, testosterone, and the estrogens.

The ability of ACTH to produce complete remissions in the acute leukemias of children was first observed by Doctor Farber in 1949.[88,89,85] Subsequently his observations were confirmed.[90]

Both ACTH and cortisone are useful agents in acute leukemia therapy

URETHANE

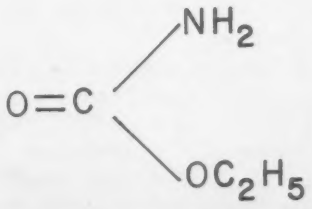


FIGURE 8

and offer some effect in the treatment of lymphosarcoma and Hodgkin's disease. In disseminated carcinomatosis, these substances do not appear to be of value.[91]

In a study of ACTH in 32 children with acute leukemia, Farber found hematological improvement in 43.8 per cent and clinical improvement in 66.3 per cent of cases. With the administration of cortisone to 42 children with acute leukemia, he reported that hematological improvement occurred in 52.4 per cent and clinical improvement in 69 per cent. As compared with the remissions produced with the folic acid antagonists, the remissions produced with ACTH and cortisone are short and vary from two to 36 weeks. Complete remissions last approximately 2 to 10 weeks. Because of these short remissions, these hormones should be reserved for the extreme and acutely ill patient. At present, these hormones are also of value in their use by sequence with the folic acid antagonists and 6-mercaptopurine, where further prolongation of the survival time in the acute leukemia of children occurs.

ACTH is administered intramuscularly, in daily doses of 60 to 200 mg., using the smaller dosages in children and, intravenously, from 25 to 50 mg. daily. Cortisone is given in dosages of from 100 to 300 mg. orally, daily, in divided dosages.

The undesirable side effects which may occur with these hormones in these dosages are sodium retention, alkalosis, hypokalemia, weight loss, edema, high blood pressure, hirsutism, acne, and psychic changes.

In the treatment of carcinoma of the prostate, androgen control therapy, consisting of bilateral orchidectomy and/or the administration of estrogens, has become fairly standard practice.

The significant stimulus following reports on the effects of castration in different diseases, including cancer, was the successful application by Huggins and his co-workers of castration in the treatment of advanced cases of carcinoma of the prostate.[92]

With androgen control therapy in carcinoma of the prostate, relief of bone pain, improvement of anemia, decrease in the serum acid and alkaline phosphatase, and pathological changes in the tumor occur. In 1946 Huggins reported on his five-year results of orchiectomy in carcinoma of the prostate. In this series of 20 patients, 18 showed improvement and, after five years, four were alive.[93] In a series of untreated or surgically treated patients with carcinoma of the prostate, only 1 to 14 per cent survived five years.

Androgen control of therapy is also of value in disseminated carcinoma of the breast in men. In these cases, marked regressions in tumors may occur.

In disseminated cancer of the female breast, both estrogen and testosterone therapy have been of value. In 1916, Lathrop and Loeb demonstrated that castration of female mice below the age of six months leads to a very marked decrease in the cancer incidence of these

animals.[94] Lacassagne, following studies on the pathogenesis of adenocarcinoma of the breast, proved that cancers may be produced in animals receiving prolonged injections of oestrone.[95]

In 1951, the subcommittee on steroids and cancer of the committee on research of the American Medical Association Council of Pharmacy and Chemistry compiled data on 886 cases of advanced mammary cancer treated with the steroid hormones.[96] In this study, they concluded that hormonal therapy is palliative only, that the best curative measure is early diagnosis and surgery. The mean duration of improvement of soft tissue tumor masses from carcinoma of the breast treated with either testosterone or estrogen is about 10 to 12 months. For bony lesions, testosterone will produce a mean duration of improvement of about 11 to 12 months, and estrogen will produce a mean duration of improvement of 16 to 18 months. The mean survival time of all patients with carcinoma of the breast in this study is 8.5 months for testosterone and 9.8 months for estrogen. Patients who showed improvement survived twice as long as those who did not. The histologic examination of biopsy and autopsy material did not reveal any changes in tumor cells or stroma which would suggest an explanation of the mechanism of action.

In regard to the choice of testosterone or estrogen in the therapy of breast carcinoma, one point is particularly important. Estrogen must not be employed in premenopausal or menopausal patients, because it may accelerate rather than inhibit the neoplasms in these cases. In the premenopausal female, testosterone propionate is the hormone of choice. The average dosages employed are 50 to 100 mg. intramuscularly, three times weekly. In women five years or more postmenopausal, estrogens are the hormones of choice for soft tissue metastasis. When bone metastasis and pain are present, testosterone appears symptomatically more effective.

The minimal effective total dose of testosterone propionate appears to be three gm. Patients should be treated at least three months before it is concluded that they will not respond.

The minimal total dose of diethylstilbesterol, dienestrol, and premarin is approximately four gm. over a period of three to six months. For ethinyl estradiol and estradiol dipropionate, the minimal dose is 200 mg. over a three to six month period.

The undesirable side effects from testosterone therapy include virilism, hirsutism, deepening of the voice, acne, and edema. With estrogen, the usual side effects of nausea, vomiting, edema, hypercalcemia, and bleeding, may appear.

Because of the lack of a close correlation between the results of chemotherapeutic agents in animal and human tumors, we have approached the selection of a chemotherapeutic agent for human tumors by the study of human tumors *in vivo* and *in vitro*.[97] Our results, with the use of this approach, have thus far been encouraging, and they

indicate a possible correlation between the effect of a chemotherapeutic agent on human neoplastic tumors in tissue culture and the effect of the same chemotherapeutic agent used clinically in the patient with advanced neoplastic disease from whom the biopsy for culture was taken. Doctor Cobb will discuss our additional studies on this problem in the following paper. Further research along these lines in a larger series of cases with different agents is imperative.

One of the thorny problems of interest to those in the field of chemotherapy is the fact that some patients with acute leukemia, Hodgkin's disease, lymphosarcoma, and carcinoma of the breast will temporarily respond to therapy with chemicals, while other patients with the same diseases, the same pathological picture, the same clinical picture will not respond. This finding leads us to feel that we may need to develop a new classification of tumors based on their biological response to chemotherapeutic compounds.

Some chemotherapeutic agents, such as urethane, the nitrogen mustards, and triethylene melamine, and hormones such as the estrogens, X rays, and radium, which are used to treat certain tumors, also generate tumors, and some give rise to genuine cell mutations.

In conclusion, while there is still no cure for disseminated neoplastic disease in man, significant progress has been made in this direction with the use of chemical agents and hormones.

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SECTION OF PHYSICS AND CHEMISTRY

TISSUE CULTURE OBSERVATIONS OF THE EFFECTS OF CHEMOTHERAPEUTIC AGENTS ON HUMAN TUMORS*

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Chemotherapeutic investigations of triethylene melamine and Puromycin were undertaken as part of a correlative program involving their clinical use in patients with disseminated or advanced neoplastic disease. The following data represent observations of 32 TEM exposed tissue culture series and 10 culture series exposed to Puromycin.

The tissue culture laboratory received sterile surgical or autopsy material from cancer patients in the hospital, and fragments of the tissue were cultured on Maximow slides, or in Gey or Porter roller tubes according to Cameron's procedures.[1]

Cultures studied in all experiments were primary, growing cultures, prior to subculture or prolonged cell maintenance. As such, they had mixed cell populations, composed of both normal and malignant cells, displaying varied types of cellular activity and growth patterns. The use of such cultures has value in that one can observe drug action on cells only just removed from the patient, before *in vitro* modulation phenomena have had a chance to occur. Disadvantages in the study of such cultures are recognized, but tissues from patients often did not lend themselves to continued subculturing. Long-term maintenance of hardy cultures was impossible to consider due to limitations of laboratory space and equipment.

Triethylene Melamine Studies

Studies on the direct action of triethylene melamine on cells in tissue culture were begun in 1950 as part of a therapy program. This compound (2, 4, 6, trisethyleneimino-1, 3, 5, triazine), recognized by Bestian in 1950 to be of value,[2] is a biologic alkylating agent with reactivity towards nucleophilic reagents similar to that of the mustards and epoxides.[3] There have been several theories postulated to explain the reason for activity. The first considers the radiationlike activity of triethylene melamine to be related to the possession of unstable 3-membered heterocyclic radicals.[4] Loveless and Ross[5] regard the ability to form carbonium ions as a more general feature of this agent rather than the ability to form compounds containing unstable ring systems. The second theory postulates carbonium ion action through a cross-

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linkage of important protein fibrous molecules, forming intracellular polymers with protein.[6] Conclusive evidence for either theory is still lacking.

In an earlier report from our laboratory, growth measurements of cultures of tissue from a series of patients with neoplastic diseases indicated that triethylene melamine completely checked fibroblasts, epithelial cells, and sarcoma cells at a concentration of 50 μg per ml. and inhibited cells at a concentration of 5 μg per ml.[7] Paterson and Boland[8] found a decrease in the mean number of mitoses in cultures of chick fibroblasts with triethylene melamine. Experiments in our laboratory,[9] using chick embryo heart fibroblast cultures established the minimal inhibitory dose (MID, *i.e.*, that concentration producing total inhibition of outgrowth as described by Pomerat[10]) as 40 μg per ml. The least injurious dose (LID) was found to be 4 μg per ml., a concentration well within the calculated therapeutic dose range *in vivo*.[11,12,13] Chick cultures exposed to this concentration showed a 14 per cent decrease in areal outgrowth, and the mitotic index was consistently lower than that of the control cultures. The drug produced scattered chromosomes at metaphase. The following series of data represent observations of the direct action of triethylene malamine on human neoplastic cells at low concentrations.

Cells of human tissue cultures varied in their response to triethylene melamine with respect to (1) specific type of cell affected, and to (2) the initial patient source material. Mitotic indexes were not determined because of mixed cell populations, the low number of observable mitoses, and the varied patterns of growth.

The effects of triethylene melamine at several concentrations are presented in TABLES 1, 2, 3, and 4. Responses of cells at the end of 24 and 48 hours were described using three terms: "toxic," indicating permanent damage as manifested by bleb formation, cytolysis, prevention of migration, and loss of cellular detail; "stasis," indicating a form of toxicity in which the cells appear normal in the unstained condition, but exhibit permanent loss of activity (such cells did not stain with routine stains); and "inhibition," indicating a temporary check of migration and mitosis. Such cultures, when subcultured or when the drug was removed, resumed growth.

There was no effect on the early wave of leucocytes, lymphocytes, and later fibroblastic overgrowth in cultures of normal adult lymph node at 4 μg per ml., the least injurious dose for chick fibroblasts (TABLE 1, Nos. 85A and 85). Triethylene melamine at 4 μg per ml. concentration inhibited fibroblasts, lymphocytes, and leucocytes in fibrosed lymph-node cultures (TABLE 1, No. 55), but was toxic to lymphocytes and relatively harmless to reticular cells of two hyperplastic lymph-node series (TABLE 1, Nos. 141 and 90). In the latter series, the extent of damage consisted of vacuolation in multinucleated giant cells (FIGURES 1

TABLE 1

CYTOTOXIC EFFECTS OF TRIETHYLENE MELAMINE ON CELLS IN TISSUE CULTURE

Exp.No.	*Tissue*	*4 μg/ml.*	*40 μg/ml.*
85	Normal chick embryo heart	Inhibition	Toxic
85A	Normal human lymph node	Lymphocytes and leucocytes—no effect. Reticular cells—no effect	Toxic
79	Normal rooster buffy coat		Toxic to macrophages
55	Fibrosed lymph node	Lymphocytes, leucocytes, fibroblasts cells—inhibition	
141	Hyperplastic lymph node	Lymphocytes—toxic. Reticular cells—no effect	
90	Hyperplastic lymph node from patient with lymphosarcoma	Lymphocytes, leucocytes—stasis. Reticular cells and giant cells—vacuolation	Toxic to all
103	Multiple myeloma mass	24 hours. Myeloma cells—no effect; reticular elements—inhibition	
93	Acute myelogenous leukemic tissue	No effect after 24, 48, 72 hours	
102	Pleomorphic lymphosarcoma mass	Slight inhibition	

TABLE 2

EFFECT OF TRIETHYLENE MELAMINE ON A SERIES OF LYMPHOSARCOMA CULTURES FROM THE SAME PATIENT

Date	*4 μg/ml.*	*40 μg/ml.*	*Clinical dose*
1/15/51	24 hours—bleb formation, disruption of solid sheet of cells. Recovery on 6th day with abundant new outgrowth. Fewer cells per area as compared to controls.		40 mg. over 6-month period
4/23/51	Initial exposure—no effect on outgrowth. Large number of multinucleated cells. Delayed cytolytic effect 15th day. Added to established cultures: vacuolation, granulation, cell death.		30 mg. since last biopsy
11/3/51	No effect.	Definite but limited outgrowth, cytoplasmic vacuolation	37 mg. since last biopsy

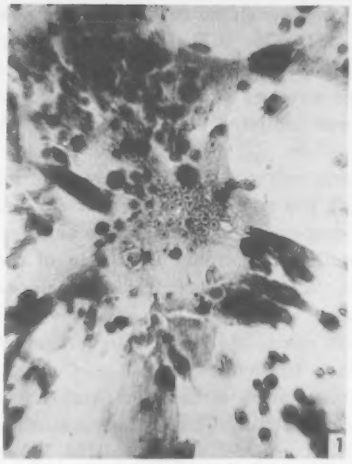
and 2). At this same concentration, multiple myeloma cells were unaffected, and many reticular elements were in abnormal metaphase (TABLE 1, No. 103). All cells of acute lymphatic leukemic lymph-node tissue were unaffected by triethylene melamine at this concentration (TABLE 1, No. 93), while lymphosarcoma cells in one series were inhibited (TABLE 1, No. 102). Observations of another fast-growing lymphosarcoma culture series (Nos. 88 a, b, c) exposed to triethylene melamine were made from three biopsy specimens over an 11-month period (TABLE 2). Four μg per ml. of the drug killed all cells after 15 days (FIGURES 2 and 3). Cells in culture were progressively less sensitive to triethylene melamine. The patient had received additive amounts of the drug during this period.

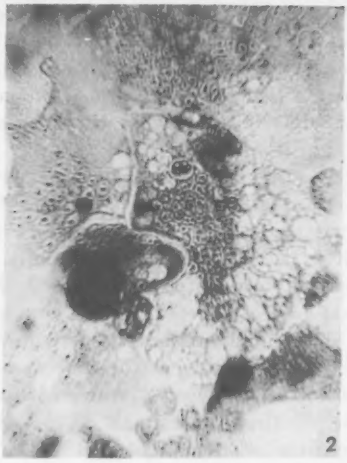
Two culture series of chronic lymphatic leukemic tissue were damaged

TABLE 3

CYTOTOXIC EFFECTS OF TRIETHYLENE MELAMINE ON CELLS IN TISSUE CULTURE

Exp.No.	*Tissue*	*4 μg/ml.*	*40 μg/ml.*
78	Chronic lymphatic leukemic lymph node	Lymphocytes and leucocytes—toxic. Reticular cells—50% inhibition. 11 days—all cells toxic	
24	Chronic lymphatic leukemic lymph node	Lymphocytes—inhibition. Reticular cells—no effect	Reticular cells—toxic
29	Reticulum cell sarcoma		24 hours. Reticular cells—50% inhibition. Lymphatic cells—Stasis
72	Hodgkin's disease lymph node		24 hours. Reticular cells—Inhibition, lymphatic cells, R-S cells—stasis
152	Hodgkin's disease lymph node	24 hrs: mitotic inhibition of reticular cells	
131	Hodgkin's disease lymph node	48 hrs: All cells—toxic	
130	Hodgkin's disease lymph node	48 hrs: stasis	
107	Hodgkin's disease lymph node	48 hrs: stasis	
51	Chondrosarcoma mass	48 hrs: toxic	
92	Fibrosarcoma & normal skin	24 hrs: sarcoma cells—inhibition. Epithelial cells—toxic	





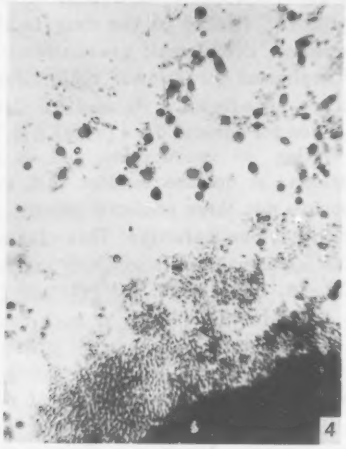


FIGURE 1, experiment No. 90. Untreated multinucleated giant cell from hyperplastic lymph node, ×400.

FIGURE 2, experiment No. 90. Same cell type exposed to 4 μg/ml. TEM for 14 days, ×400.

FIGURE 3, experiment 88B. Lymphosarcoma control, ×200.

FIGURE 4, experiment No. 88B. Fifteenth day after exposure to 4 μg/ml. TEM, ×200. All cultures fixed and stained with May Grünwald-Giemsa.

after exposure to 4 μg per ml. and 40 μg per ml. concentrations respectively of triethylene melamine (TABLE 3, Nos. 78 and 24). The reticular cells were relatively less affected. Five culture series of Hodgkin's disease lymph nodes showed varied response to triethylene

melamine. Four of the series were very sensitive to the drug at low concentrations (TABLE 3, Nos. 152, 131, 130, 107). Reticular cells in one culture series were inhibited at 40 μg per ml. and lymphatic cells were killed (TABLE 3, No. 72). Large myeloma cells were not damaged by triethylene melamine but mitosis was inhibited in reticular elements (TABLE 3, No. 103).

Fast-growing cells in chondrosarcoma cultures from autopsy material were cytolyzed after exposure to 4 μg per ml. of triethylene melamine (TABLE 3, No. 51). Sarcoma cells in drug-exposed fibrosarcoma cultures were inhibited only, but epithelial cells, presumably from skin of the patient, were killed (TABLE 3, No. 92). Control cultures developed fast-growing sheets of normal epithelium.

Tissue cultures from 14 patients with carcinoma showed no consistent response of malignant or stromal cells to triethylene melamine. Cultures of rectal carcinoma tissue and lung carcinoma metastatic skin tissue were noticeably resistant to low concentrations of triethylene melamine (TABLE 4, Nos. 114 and 144). Carcinoma cells were killed, giant mesenchymal cells unaffected in breast carcinoma tissue (No. 118). Cytotoxic effects of the drug in this series were manifested by bleb formation, cytoplasmic granulation, vacuolation, and occasional pyknosis of malignant or stromal cells, depending upon the individual patient source (FIGURES 5, 6, and 8). Often visible damage by the drug was delayed for several days (TABLE 4, Nos. 149 and 115).

During an examination and an evaluation of tissue culture observations, it became evident that the response of cells in culture fell roughly into three artificial groups, according to their relative sensitivity to triethylene melamine. This classification is given in TABLE 5. There was apparent correlation with *in vivo* clinical observations in Nos. 131, 130, 88, 107, 78, 24, 90, 144, and 29. Additional studies are in progress to determine the validity of such correlation *in vivo* and *in vitro*.

To summarize, exposure of mixed-cell populations of human neoplastic tissue in culture to 40 μg and 4 μg per ml. of triethylene melamine demonstrate the direct action of the drug on cells. Studies revealed a variation in the type of cell affected, dependent upon the original tissue source. The least injurious dose of triethylene melamine for chick fibroblast cultures, namely 4 μg per ml., produced no change in normal adult lymph-node cultures, lung metastatic tissue, rectal carcinoma cells, and an acute lymphatic leukemic lymph node. At this same concentration, permanent damage occurred to cells in three Hodgkin's disease series, a chondrosarcoma, and a metastatic carcinoma culture series.

Puromycin Studies

The antibiotic Puromycin, isolated from *Streptomyces albonigęr*, and found active against certain bacteria and trypanosomes[14] has been tested against solid tumors for carcinostatic action by W. Troy and his

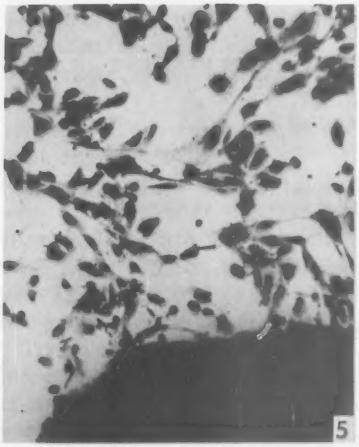
TABLE 4

CYTOTOXIC EFFECTS OF TRIETHYLENE MELAMINE ON CELLS IN TISSUE CULTURE

Exp.No.	*Disease*	*4 μg/ml.*	*40 μg/ml*
95	Carcinoma metastasis to lymph node		24 hrs. stasis
42	Metastatic hypernephroma	Initial exposure: meager outgrowth of carcinoma cells	24 hrs. 100% cytolysis
36	Carcinoma metastasis to lymph node		Initial exposure: lymphocytes & leucocytes, unaffected carcinoma cells—toxic
144	Lung carcinoma metastasis to skin	No effect	
123	Carcinoma metastasis to lymph node		Inhibition of reticular cells & carcinoma cells
122	Fibroareolar tissue with anaplastic carcinoma cells	48 hrs: mitotic inhibition of fibroblasts. Carcinoma cells—stasis	48 hrs: cytolysis—all cells
118	Breast scirrhous carcinoma tissue		48 hrs: toxic to carcinoma cells. Giant mesenchymal cells unaffected
150	Myofibroma of the uterus	24 hrs: mitotic inhibition of fibroblasts	
149	Carcinoma metastasis to lymph node	Fibroblast inhibition. 4 days: increased cell debris, pyknosis	
136	Carcinoma metastasis to lymph node	Cytolysis 70% of all cells	
139	Carcinoma metastatic mass taken from peritoneum	48 hrs: granularity, pyknosis	
113	Breast carcinoma metastatic mass	24 hrs to 72 hrs—no effect on carcinoma cells; 7 days—30% reticular cells rounded. Inhibition	
115	Carcinoma metastasis to liver	24 hrs: inhibitory, renewal of growth after transfer. Toxic after 5 days continuous exposure	
96	Adenocarcinoma metastatic mass from peritoneum	24 hrs: mitotic inhibition of fibroblasts, cytoplasmic vacuolation of carcinoma cells	
114	Primary rectal adenocarcinoma mass	No effect	Toxic to all cells

co-workers[15] and J. J. Oleson and co-workers[16] with only mild success. It was carcinostatic against a mammary adenocarcinoma of CH_3 mice and the mouse mammary adenocarcinoma 755 of C57 BL mouse, but only mildly inhibitory against a mouse *glioblastoma* growing in the chick embryo yolk sac and against transplanted mouse sarcoma 180. Toxicity developed in rats with tumors receiving high levels of the compound for five to six weeks.

Puromycin administered to 50 patients with disseminated or advanced



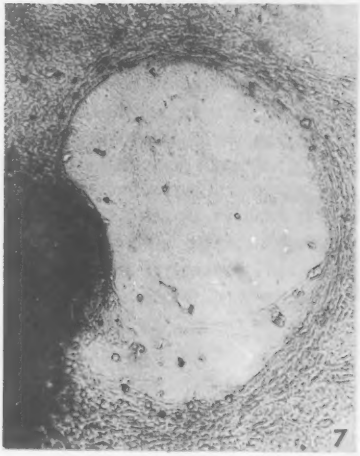


FIGURE 5, experiment No. 42. Carcinoma control culture, ×100. May Grünwald-Giemsa.

FIGURE 6, experiment No. 42. Carcinoma cells exposed to 4 μg/ml. TEM for four days, ×100. May Grünwald-Giemsa.

FIGURE 7, experiment No. 92. Living 14-day roller-tube culture showing normal epithelial sheet, ×60.

FIGURE 8, experiment No. 92. Culture seven days following 4 μg/ml. TEM exposure for 24 hours, ×60.

incurable neoplastic disease gave only slight to moderate objective evidence of tumor regression in 14 of the 50 patients.[17]

Puromycin (C_{22} H_{29} N_7 O_5) is a diacidic base consisting of three components: (1) 6-dimethylaminopurine; (2) o-methyl l-tyrosine; and

(3) D-3-aminoribose. Eichorn found evidence of Puromycin cytotoxicity in tissue-culture studies.[18] Additional tests *in vitro* by Eichorn indicated that certain combinations of the analogues of these fractions were active. Data reported here include investigations of the direct effect of the intact Puromycin molecule on nine culture series of human neoplastic tissue and one spontaneous mouse tumor. The outgrowth usually consisted of malignant cells together with stromal cell types. A list of the tissues studied *in vitro* is presented in TABLE 6.

Initial dose-level experiments were necessary due to lack of any previous tissue culture data for Puromycin. Exposure of cells to a range of concentrations in culture revealed several cytological effects. These effects are described in the following paragraphs.

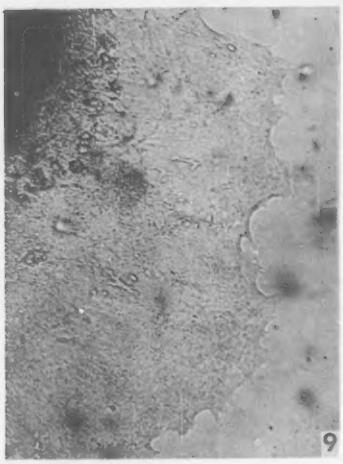
Forty Micrograms per Milliter

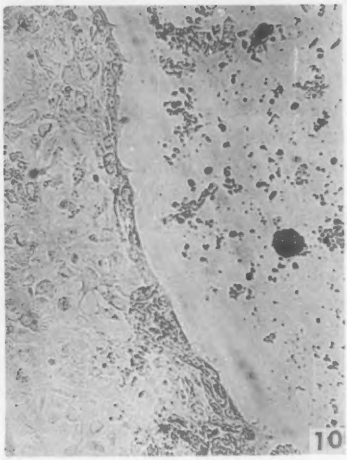
(1) Thirty-minute exposure followed by drug removal: (a) one hour

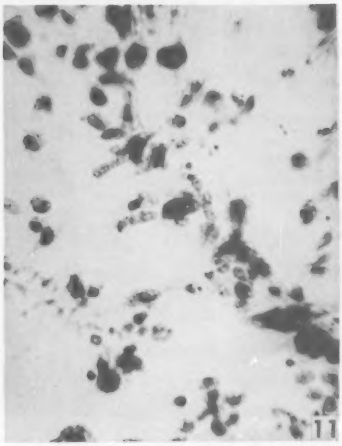
TABLE 5

SUMMARY OF CYTOTOXIC EFFECTS OF TRIETHYLENE MELAMINE

	Resistant to TEM
29	Hodgkin's disease lymph node
72	Hodgkin's disease lymph node
93	Acute myelogenous leukemic tissue
85A	Normal lymph node
102	Pleomorphic lymphosarcoma
42	Metastatic hypernephroma
113	Breast carcinoma metastatic mass
144	Lung carcinoma to skin
123	Carcinoma lymph node
	Moderately Sensitive to TEM
92	Fibrosarcoma
141	Hyperplastic lymph node
55	Fibrosed lymph node
90	Hyperplastic lymph node
96	Adenocarcinoma metastatic mass
103	Multiple myeloma mass
150	Myofibroma of uterus
95	Carcinoma metastasis to lymph node
36	Carcinoma metastasis to lymph node
118	Breast scirrhous carcinoma tissue
149	Carcinoma metastasis to lymph node
115	Carcinoma metastasis to liver
	Sensitive to TEM
152	Hodgkin's disease lymph node
131	Hodgkin's disease lymph node
130	Hodgkin's disease lymph node
107	Hodgkin's disease lymph node
51	Chondrosarcoma
78	Chronic lymphatic leukemic mass
92	Normal skin epithelial cells
122	Fibroareolar & carcinoma metastatic tissue
136	Carcinoma metastasis to lymph node







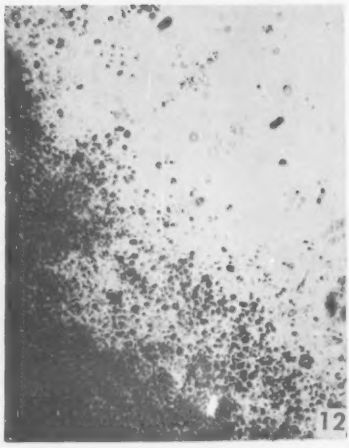


FIGURE 9, experiment No. 129. Living mouse adenocarcinoma control culture, ×60.

FIGURE 10, experiment No. 129. Culture 24 hours after a 30-minute exposure to 40 μg/ml. Puromycin, ×100.

FIGURE 11, experiment No. 130. Control Hodgkin's disease reticular and lymphatic cells, ×400. May Grünwald-Giemsa.

FIGURE 12, experiment No. 130. Hodgkin's disease tissue culture exposed for five days to 40 μg/ml. Puromycin, ×400. May Grünwald-Giemsa.

after exposure—small number of pyknotic cells, nuclear membrane shrivelled but nucleolus still intact, in epidermoid carcinoma cultures (TABLE 6, No. 121); (b) 24 hours later—cytolysis of all epithelial cells, few

TABLE 6

TISSUE CULTURES USED FOR PUROMYCIN STUDY

Exp. No.	*Tissue Classification*
121	Epidermoid carcinoma plus stromal cells
126	Epidermoid carcinoma plus stromal cells
127	Multiple myeloma mass
129	Mouse spontaneous mammary adenocarcinoma
130	Hodgkin's disease lymph node
139	Breast adenocarcinoma tissue
141	Hyperplastic lymph node
142	Lymphosarcoma lymph node
144	Metastatic hypernephroma
152	Hodgkin's disease lymph node

damaged fibroblasts as seen in cultures of the same tissue, increased cell debris, cytolysis of individual cells in the solid epithelial sheets of mouse adenocarcinoma cultures (FIGURES 9 and 10); (c) 48 hours—total cytolysis (No. 121) in epidermoid carcinoma tissue; (d) five days—complete cellular disintegration and loss of staining ability in Hodgkin's lymph-node cultures (Number 130, FIGURES 11 and 12).

Exposure to 40 μg per ml. of Puromycin for 30 minutes caused irreversible cell damage to cultures of epidermoid carcinoma, mouse mammary adenocarcinoma and Hodgkin's disease reticular cells.

(2) Continued Puromycin exposure: (a) 24 hours—50 per cent stromal cells disintegrated, rest granular in epidermoid carcinoma tissue (No. 126), multiple myeloma cultures had 30 per cent of myeloma cells

FIGURE 13, experiment No. 126. Untreated epidermoid carcinoma stromal cells, ×400.

FIGURE 14, experiment No. 126. Stromal cell exposed to 4 μg/ml. Puromycin for 24 hours, ×400. May Grünwald-Giemsa.

swollen, with blebs and loss of internal structure, remaining fibroblasts filled with clear refractile droplets (No. 127); (b) 48 hours—stromal cells of epidermoid carcinoma were altered to granular bleblike remnants of original cells (No. 126); all cells in myeloma cultures were totally destroyed with subsequent loss of staining ability (No. 127).

Ten Micrograms per Milliliter

(1) Continued exposure: 48 hours—clusters of granular epithelial cells in open liquefaction pool, and no recovery 48 hours after drug removal in epidermoid carcinoma cultures (No. 121).

Four Micrograms per Milliliter

(1) Continued exposure: 24 hours—formation of a large single, cytoplasmic vacuole enclosing a mass of eosinophilic material, and no visible alteration in nucleus, nucleolus, or cytoplasmic processes in stromal cells of epidermoid carcinoma cultures (No. 126, FIGURES 13 and 14).

Two Micrograms per Milliliter

(1) Twenty-four hours—50 per cent of malignant epithelial and stromal cells of culture from carcinoma skin metastasis extended but processes beady, and 50 per cent of cells rounded, cell-membrane collapsed and irregular. Irreversible cell destruction after 40 hours of exposure (No. 144).

(2) Forty-eight hours—cytoplasmic granulation in carcinoma cells (No. 139, breast carcinoma tissue). Over 50 per cent of cells rounded or blebbed, cytolysis typical in stromal cells from a hyperplastic lymph node. All malignant and stromal cells of Hodgkin's lymph node were in various stages of disintegration. No recovery 48 hours after Puromycin removal (No. 152).

(3) Seventy-two hours—no resumption of growth of any cultures.

One Microgram per Milliliter

(1) Forty-eight hours—fast growing stromal cell cultures from a lymphosarcoma mass were totally unaffected. Normal mitotic stages were present in drug exposed and control cultures (No. 142).

To summarize, data accumulated for Puromycin-exposed tissue cultures indicated irreversible damage to cells at 40, 10, 4, and 2 μg per ml. concentrations. There was a lack of preferential toxicity for specific cell types and abnormal mitoses were not observed. Toxicity was manifested by bleb formation, cytolysis, and loss of staining ability. Stromal cells of multiple myeloma cultures (No. 126) exposed to 4 μg per ml. of Puromycin formed single giant vacuoles which enclosed eosinophilic material.

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SECTION OF BIOLOGY

THE CYTOANALYZER—AN EXAMPLE OF PHYSICS IN MEDICAL RESEARCH*

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Introduction

The purpose of this paper is to discuss briefly an example of the application of physical principles and methods to a problem of biological origin. We are familiar with elegant examples of physical instrumentation—the optical and electron microscope, the spectrometer, the microbalance—all employed extensively in biological investigations.

The problem at hand is the provision of a high-speed aid to cytology staffs for the automatic screening of normal from abnormal smears of exfoliated cells, a problem not solely of instrumentation, but one of great breadth of investigation and development as well. The provision of such an aid is aimed at increasing the productivity of current cytology staffs sufficiently to allow the possibility of mass screening for cancer, an aim unattainable by present methods.

The one underlying basic premise of the approach to be outlined assumes acceptance of present-day methods of exfoliative cytology, as employed so widely and successfully, as the starting point of the problem. This initial condition is adopted clearly as a practical matter, allowing the use of the vast resources of case history material, supplementing, and not vitiating, the extensive training of large numbers of cytologically educated personnel, and permitting current cooperative action between the cytological laboratories and these investigations.

There are four distinct problem areas within the over-all objective:

(1) The determination of the fundamental quantitative differences between normal and abnormal exfoliated cells of a form compatible to techniques of high speed measurement.

(2) The design of optimum physical instrumentation to convert the optical-spatial information of the smear into a form adaptable to modern methods of data handling and processing.

(3) The design of computing equipment to accept the incoming data and to sort it according to the cytological data rules, to make the necessary measurements, and, finally, to perform the act of decision as to the classification of the smear as normal or abnormal.

(4) The engineering of the equipment to meet clinical requirements without exceeding feasible economic dimensions.

It is clear, then, that this objective, in its entirety, is a system, and

*This paper was the first of two papers presented at a meeting of the Section on December 13, 1954.

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that no one problem is independent of the others. The efforts directed toward each problem must be made in recognition of the methods and approaches used on the others. This approach expresses, in this particular framework, the sense of the concept of modern system design. While this concept is not particularly pertinent to pure research, it is most availing and efficient when the objective is the actual provision of a piece of equipment to fulfill a given need. Thus, the classical freedom of pure research is subordinated here to the system requirements of a coherent, internally compatible program of research, development, and engineering.

As this project is still in the introductory state, the following discussion will show the general approach being taken to the first three problems, indicating the strong influences of modern physics and electronics on this most important biological problem.

The Cytology

Qualitative and comparative literature[1,2,3,4] describing the differences between normal and cancer cells is copious and effective. Out of this literature come the statements that cancer cells, in exfoliated smears of the vagina and cervix, distinguish themselves from the normal cells in the same smear by having: (1) larger nuclei; (2) more deeply stained nuclei; and (3) a smaller cytoplasm–nuclear ratio.

Each of these characteristics can be measured quantitatively. The literature shows several studies giving quantitative values of these variables separately. Data of the nucleic acid content of the nuclei, or a quantity proportional to it, have been published.[5,6] An association between nuclear size and nucleic acid has been shown.[7] Data have been given on the relationship between the C/N ratio and cell size for cells of less than 800 μ^2.[8] Data showing the simultaneous values of cell size, nuclear size, and nuclear density have been reported[9,10] and, being self-correlated, present coherent patterns of cell behavior under normal and abnormal growth conditions.

The foregoing numerical information on cells, though incomplete in many respects, has body enough today to allow us to set up rough quantitative bounds of discrimination in the three dimension domain of nuclear density, nuclear size, and cell size. The acquisition of this type of quantitative information on the cells and cell growth patterns, across the wide spectrum of physiological conditions exhibited in the female generative tract, is going ahead to provide definitive quantitative rules of discrimination between normal and abnormal cells without which no automatic aid can be effective.

The Cytoanalyzer

FIGURE 1 shows our present model of the cytoanalyzer. It is the third in a series of laboratory models, the first being a scanner for

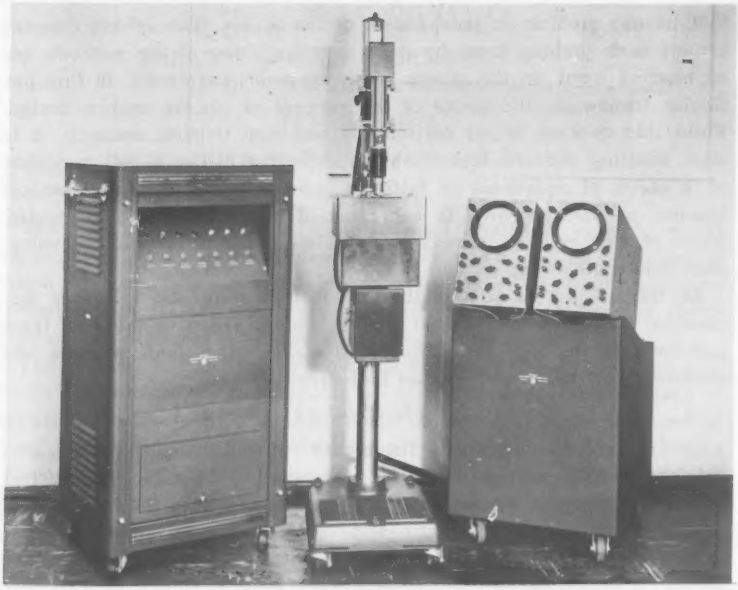

FIGURE 1

fluorescent stained smears with only crude amplitude measurement capabilities, and the second being a scanner for visible, conventionally stained Papanicolaou smears with somewhat better data-reading capabilities. The present model possesses even better data-handling equipment, and has a modicum of automatic functions.

The unit on the left is the power supply and computer. The center unit is the scanner, and the oscilloscopes, on the right, are for data-monitoring and presentation.

This model serves principally as a vehicle for data gathering and for trial and evaluation of the various physical and electronic elements of the system in its current development.

The present over-all rate of reading is about 50 $mm.^2$ per minute, requiring 12 minutes to read all of the 600 $mm.^2$ of a conventional smear. An analysis of the signal to noise situation shows that this speed can be increased by at least a factor of 10 when deemed desirable. If, on the other hand, the cells of these smears were ideally distributed, 10,000 cells could then be scanned in 30 seconds. This possibility points up the gains which can be made with improvements of smear preparations.

The scanner. The scanner performs the function of converting the density field of the slide into a serial electric current which is then used to analyze these several cell properties.

The illuminating system is conventional tungsten Köhler illumination of the field of the 45 × objective of a microscope. The real image is scanned by a synchronous driven disc with 30 equiradial apertures, the diameters of which are 5 μ when referred to the object plane, and which turns at a rate of 30 revolutions per second. Thus, 900 lines of scan per second are generated. The length of a scan line is 250 μ.

The stage is driven at a constant velocity corresponding to 4 μ advance for each line of scan. Automatic programming of both the x and y transports allow unattended examination of the entire slide.

The light, penetrating the specimen and the scanning apertures, is projected on a photomultiplier by an optical system antisymmetrical to the illumination system.

FIGURE 2 shows diagramatically the conversion of the density field to an electrical current.

From the preceding numerical values one sees that the duration of one line of scan is approximately one one-thousandth of a second. The smallest nucleus we can measure here will give a signal of about 40 millionths of a second. A bandwidth greater than 100 kilocycles is currently employed. The continuous nature of the scanning process used here allows the employment of a high duty factor of reading.

The Computer. The basic function of the computer is to accept the signal from the scanner, to apply certain rules of admission or rejection to the signals (based on quantitative cytological data), to measure the

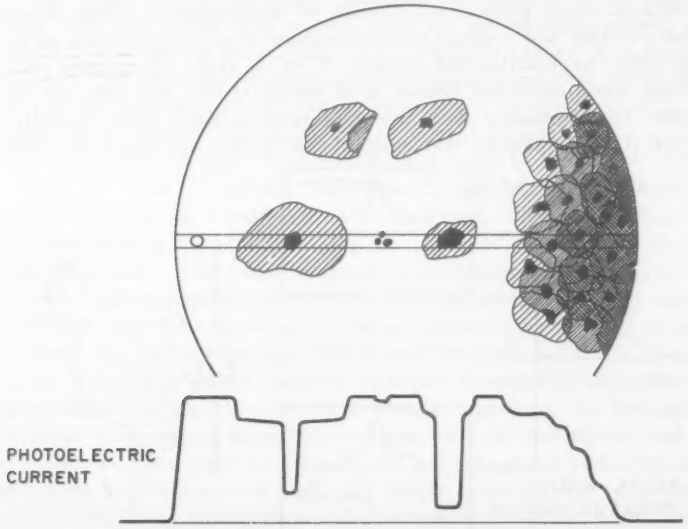

FIGURE 2. Photoelectric current of one line of scan.

desired cell characteristics and to distinguish between signals arising from abnormal cells and signals due to normal cells.

FIGURE 3 shows the elementary functions of the mensuration and recording channels for handling nucleus data. The signal from the photomultiplier is fed to the amplitude channel which measures absorption. This channel is simply a peak-reading voltmeter, the voltage of which is applied continuously to the vertical plates of an oscilloscope. The electron beam of the oscilloscope is normally biased off, and the voltage reading is not entered until the rule-making circuits dictate that this value should be entered.

The width of the nucleus is read by an integrating circuit which produces an output voltage increasing linearly with time, being turned on by the nucleus absorption signal exceeding a given value (A_1) and being turned off when the signal returns to this level. Its value is stored awaiting entry on the horizontal axis of the oscilloscope by the rule circuits.

It is thus seen that, when the electron beam is pulsed on, the nuclear absorption and width will be simultaneously entered as a dot of light on the oscilloscope. A permanent record of thousands of entries can be made on one frame of an open shutter recording camera.

With the exception of excluding signals less than A_1, the measurement and recording is of continuous analogue form of high resolution.

FIGURE 4 shows the elements of a fairly elementary digital computing circuit whose function is principally negative, in that it excludes from the data-recording portion all signals of nondiagnostic value. Since routine cervical and vaginal smears contain much density data of no value, such as multilayered clumps of cells, trails of mucous with entrained blood cells and debris, it is essential that this data not be recorded to intermingle with the data from isolated cells. Finally, cytological data indicate that most of the normal and abnormal cells

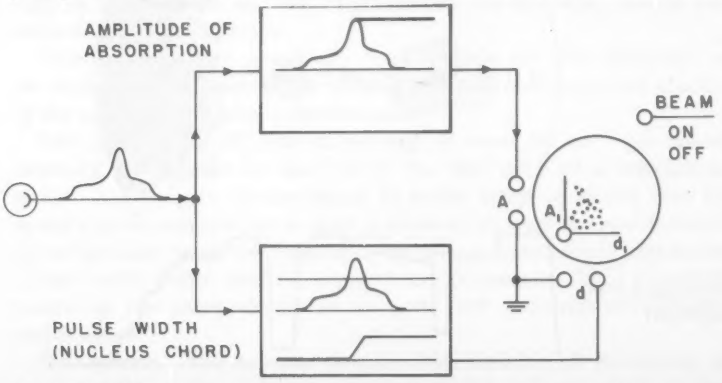

FIGURE 3. Nucleus absorption and size measurement channels (analogue).

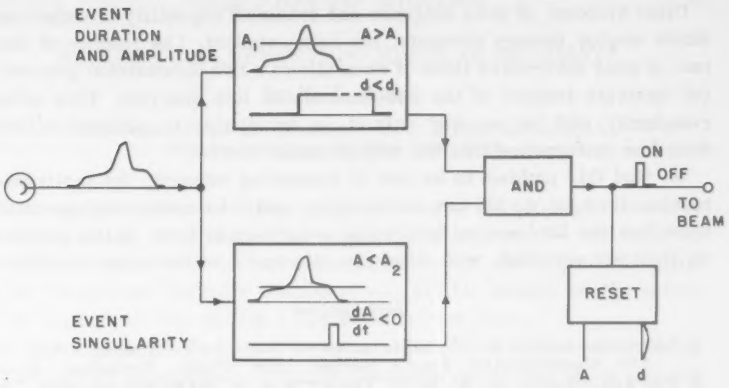

FIGURE 4. Channels of definition, acceptance, rejection (digital).

of interest have diameters of less than 80 micra, and nuclear diameters of less than 30 micra.

These data allow the setting of several useful exclusion rules. The upper part of FIGURE 4 indicates that the occurrence of a nucleus signal exceeding a given value of absorption A_1 initiates a pulse of constant height and fixed length—45 micra in the present case. Any highly absorbing event can start this pulse gate. The signal is fed to the logical "and" circuit.

The lower part of the figure indicates the carrying out of one more rule: that the signal from a cell shall not be measured unless it is isolated and free of other cells. This condition can be recognized by the fact that when the scanning aperture leaves a cell, the signal drops to the zero absorption level. Thus, by setting a threshold level A_2 and requiring a negative derivative at this point, this rule can be invoked. The occurrence of such an event sends a pulse to the "and" circuit.

Both of these conditions (condition 1, *and* condition 2) must be achieved before the "and" circuit is energized, which then causes the electron beam to enter the data on the oscilloscope face. Having done this, the "and" circuit also energizes a reset pulse which sets to zero the data in the measurement channels, clearing them for the next cellular encounter. All this computation is being performed as the cell is being scanned; *i.e.*, in less than one fifth of a millisecond.

The description of the computer thus far, is meant to illustrate how quantitative cytological rules from research data may be set into electronic elements to allow the acceptance of desired signals and the rejection of undesired ones. One should not take these particular operations to be sufficient or final, but simply operations we are testing now for adequacy of performance. The foregoing illustrates a pure serial system, one which operates and makes decisions at the same rate as the inflow of data.

Other systems, of more elegance and enhanced capability of definition, which employ storage elements, are being studied. The memory of one, two, or more successive lines of scan allows a two-dimensional geometrical analysis instead of the one-dimensional line analysis. This added complexity will be required only if we are unable to achieve reliable error-free performance from the simpler serial system.

We find this problem to be one of consuming interest, the preliminary results, thus far, to be very encouraging, and, of course, we have every hope that the fundamental biological and physical facts of the problem, as they are revealed, will allow the attainment of the prime objective.

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SECTION OF BIOLOGY

RECENT DEVELOPMENTS IN CELLULAR MICRURGY*

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Micrurgical studies on living cells have been further implemented by the development of a volumetric submicromanipulator,[1] the microdilatometer,[2] and the ultraviolet micromanipulator[3] with which invisible ultraviolet images are instantly translated into visible images on the screen of the control monitor through a closed television chain.

A new approach towards the transplantation of subcellular particulates, cytochemistry, and single-cell cultures has been developed which requires the transfer of cells from their normal aqueous environment to a nonaqueous, nontoxic fluid. The essential aspects of this new approach will be described in this report.

The injection of aqueous and nonaqueous fluids into cells. Numerous studies have shown that the injection of a given volume of aqueous solution into a cell generally disturbs a larger volume of cytoplasm. In addition to the traumatic effects of the injection, there may be some diffusion of the injected substances to other parts of the cell with the possibility of additional injury. Solutions containing a "balanced" salt mixture generally produce less damage and recovery from such injections is more rapid. Usually, the larger the amount injected, the greater is the injury to the cell.[4,5] Cells are able to tolerate certain salt mixtures, if such mixtures are injected slowly while, if the same mixture is injected rapidly, the injury may be severe and frequently irreversible.

On the other hand, the injection of innocuous nonaqueous fluids produces essentially no injury to the cell. The volume of cytoplasm displaced by the injection of an oil drop is equivalent to the volume of the oil. The trauma produced in *Asterias* oöcytes, for example, is considerable less than the volume of cytoplasm displaced, as shown by drop-retraction methods.[6] In this procedure, the oil drop is drawn back into the micropipette until the drop now approximates one tenth to one fifth its original diameter. On continued retraction of the drop, a cytolytic zone no larger than a small fraction of the volume of injected oil becomes visible. The fact that the volume of the traumatized cytoplasm is smaller than the oil drop suggests that the cytoplasmic framework is elastic and

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that, on removal of the stress, the cytoplasm returns to its former pattern exclusive of the damaged portion.

Except for the toxic oils, which may produce local or general cytolysis, there are no further cytoplasmic changes surrounding the oil drop. There are no indications of diffusion away from the boundary between oil and protoplasm. The volume of injured cytoplasm is thus limited by the volume of the injected fluid, and the zone of action, if any, is limited to the interfacial area between the oil and cytoplasm.

These results confirm findings indicated by other data, that the rapid injection of aqueous solutions causes considerable injury to cytoplasm. By contrast, equally rapid injections of an equivalent volume of innocuous, nonaqueous fluids rarely have any drastic action on cytoplasm. *Arbacia* and *Lytechinus* eggs will cleave, even though an oil drop may have been injected into the wall of the cleavage furrow.[7] The injection into the furrow of an equivalent, or smaller volume of any known aqueous solution blocks cleavage, an effect frequently irreversible.

The violent disruption of cells in various media, as occurs during the preparation of homogenates, exposes the subcellular particulates to large volumes of incompatible environments. Kassel's mixtures,[4,5] when injected into amoebas, are the least toxic of all aqueous media tested. On the other hand, the transfer of a nucleus from the amoeba to Kassel's solution, so readily performed with a microneedle, causes slight but nevertheless appreciable changes in the morphology and basophilic components of the nucleus.

Until the proper balance between ions and other substances is achieved, some damaging effects on cytoplasmic structures following the microinjection of aqueous fluids may always be expected. This report, which summarizes the differences between the actions of aqueous and nonaqueous fluids, is intended to show that water is a toxic substance, and that all aqueous solutions so far known will manifest a degree of toxicity beyond that induced mechanically as a result of the injection. Obviously, if one could match the aqueous phase of protoplasm, these damaging effects of aqueous injections should be no greater than that induced by injections of inert, nonaqueous fluids.

Unfortunately, we do not have enough information concerning the composition of the aqueous phase of protoplasm. A further complication is that the proper aqueous mixture of salts and other components required to obviate injury to cellular structures may have to be especially designed for each cell type—and on the basis of past experience, this task would be a formidable one. It is possible, however, to gain certain valuable information on the distribution of enzymes, as well as to facilitate the transfer of subcellular particulates from cell to cell, by eliminating water or aqueous media. On that basis, a further study on nonaqueous fluids should be productive.

Transferring cells from aqueous to fluorocarbon fluids. Two new groups of nonaqueous fluids are being investigated: (1) dimethylsiloxane polymers and (2) fluorocarbons. Of these two, the latter compounds, because of their high densities, low surface tensions, negligible solvent action, and chemical inertness, are of potentially greater value. The available compounds are listed in TABLE 1. Mixtures of various densities are made by combining n-heptane (density, 0.684) with either genetron or freon. These mixtures are too volatile for micrurgy, hence it is necessary to use fluorocarbons with much higher boiling points (N-43 or Kel-F No. 1 oil). Both N-43 and Kel-F oil are less volatile than water, so that hanging drops of these fluids will last for several hours. An unprotected hanging drop of Kel-F oil will survive overnight in the micrurgical moist chamber.

Cells may be conveniently transferred from their aqueous environment to fluorocarbon fluids by centrifugation, providing the density of the fluorocarbon is greater than the aqueous phase and less than the cells. For this purpose, either genetron-heptane or freon-heptane mixtures are prepared, with densities ranging from 1.04 to 1.09. The required intensity of the centrifugal acceleration, as well as the duration of the centrifugation, will vary with the cells studied. With isolated calls from vertebrate tissues, centrifugation durations of 5 to 15 minutes at 5,000 to 15,000 G are generally sufficient to transport the cells across the water/fluorocarbon barrier. When this end is accomplished, the aqueous phase is removed. Another fluorocarbon, either N-43 or Kel-F oil, is added to increase the density of the mixture to from 1.5 to 1.8. The addition of N-43 to genetron-heptane or freon-heptane mixtures almost instantly displaces most of the heptane which rises to the top and forms a separate layer. The Kel-F oil mixes well with either mixture containing heptane.

On transferring cells to the fluorocarbons, not only is the environment nonaqueous (little, if any, water is carried with the cells), but the high density of the fluid causes the cells to rise in a hanging drop, so that

TABLE 1

FLUOROCARBONS		
Compound	Formula	Density
Genetron 226*	$C_2F_2Cl_4$	1.55
Freon 112*	$C_2F_4Cl_2$	1.637
Fluorochemical 0–75**	$C_8F_{16}O$	1.760
Fluorochemical N–43†	$(C_4F_9)_3N$	1.872
Kel–F No. 1 oil‡	$(-CF_2-CFCl-)_x$	1.94

*Courtesy of Dr. A. E. Gessler, Research Laboratories, Interchemical Corp.
**Fluorated cyclic ether, courtesy of Minnesota Mining & Manufacturing Co., New Products Div.
†Heptacosafluorotributylamine, courtesy of Minnesota Mining & Manufacturing Co., New Products Div.
‡Polymer of trifluorochloroethylene, Chemical Manufacturing Division of M. W. Kellogg Co.

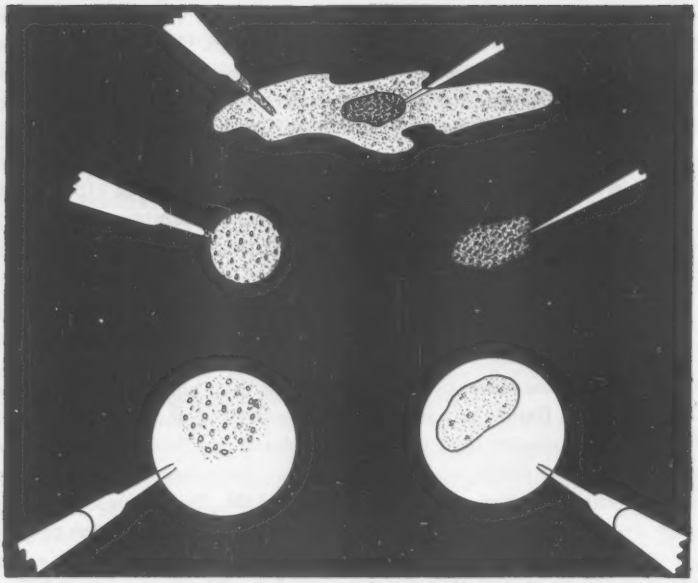

FIGURE 1. **Cell transferred from aqueous environment into fluorocarbon fluid (black). On the right side is the nucleus which was removed with a microneedle and transferred directly into the fluorocarbon fluid, while cytoplasmic particulates (left side) are removed with a micropipette and injected into the fluorocarbon. Known volumes of cytoplasm are removed with the volumetric submicromanipulator. Measured volumes of aqueous mixtures are placed into the fluorocarbon fluid, with micropipettes, to surround the isolated particulates. This procedure is useful for determining the cytochemical properties of the particulates, especially where the end product of the reaction develops a color.**

they come to rest on the lower surface of the cover glass (FIGURE 2). This position of the cells is ideal for subsequent micrurgy.

Several minor problems had to be solved before it became feasible to set up hanging-drop preparations on cover glasses. All fluorocarbons so far tested have peculiar physical properties, some of which probably stem from the relatively low surface tensions of these fluids. Both N-43 and Kel-F oil spread extensively on clean glass surfaces. After considerable experimentation, it was found that if cover glasses are first coated with a thin film of silicone stopcock grease, the spreading of these fluorocarbons is greatly reduced. A thin film is best deposited by dipping the cover glass into a solution of stopcock grease in benzene. As soon as the solvent has evaporated, the cover glass is carefully wiped with lens paper. The film of silicone that remains is sufficient to prevent extensive spreading of the fluorocarbons.

Isolating subcellular particulates. The first application of the fluorocarbon technique, illustrated in FIGURE 1, shows the extraction of sub-

cellular particulates from cells. These particulates, removed with either a micropipette or microneedle, are transferred directly to the fluorocarbon mixture which surrounds the cells. The action of aqueous mixtures may now be tested by applying them to the isolated particulates with a micropipette. The addition of certain aqueous mixtures frequently produces striking changes in the appearance of isolated nuclei. In a similar way, various cytochemical color reactions may be produced. The most useful are those dependent on enzymatic activity such as the Manheimer-Seligman[8] procedure for phosphatase activity or the Nadi reaction for demonstrating cytochrome oxidase activity.[9]

Transfer of subcellular particulates from cell to cell. A second application, FIGURE 2, is designed to facilitate the transfer of subcellular

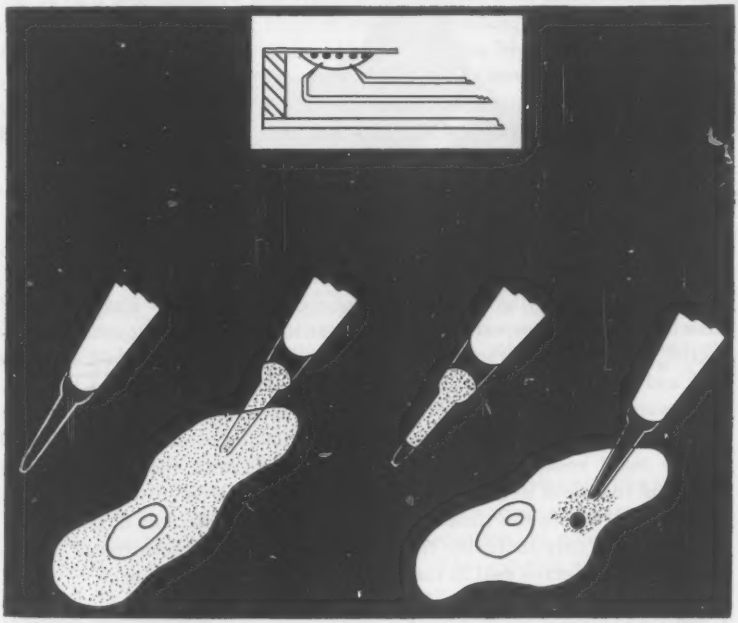

FIGURE 2. Transferring cytoplasmic particulates from cell to cell. Insert shows moist chamber with hanging-drop preparation and two micropipettes. Note position of "cells," which rise and rest against lower surface of cover glass due to the high density of fluorocarbon fluids. Cytoplasmic particulates are protected by fluorocarbon seals and siliconized micropipettes. A small amount of fluorocarbon is first drawn into the pipette (inner seal). After the pipette is inserted into a cell, a measured volume of cytoplasm is then drawn in. Immediately after pipette is removed from the cell, the tip is sealed (outer seal) with a small volume of fluorocarbon. On injection of the contents of the pipette into another cell, the outer seal is first injected followed by the cytoplasmic sample. This small volume of fluorocarbon, completely innocuous, indicates that the injection into the host cell was actually made and, furthermore, this droplet serves to mark the injected cell.

particulates from cell to cell. The elimination of water from the environment removes the most serious obstacle in such transfers. The method developed here includes the most careful insulation of the cytoplasmic sample from potentially dangerous noncellular environments. Fluorocarbon seals and siliconized micropipettes are therefore used.

From previous tests made with fluorocarbon fluids, using microinjection, it was established that neither N-43 nor Kel-F oil produces any effects on those subcellular structures that can be seen with the light microscope. It seemed reasonably safe, then, to use these fluorocarbons as internal and external seals for small samples of cytoplasm extracted with a micropipette.

Most samples of cytoplasm, when brought in contact with clean glass, will coagulate, and thus plug, a micropipette (for example, see Duryee[10]). This action not only produces obvious changes in the cytoplasm, but also prevents the transfer of such a sample to another cell. The coagulation of cytoplasm is prevented, in most instances, if the micropipettes are coated with a very thin layer of silicone. Less change is thus induced in the cytoplasmic fraction and, furthermore, such material can be expelled from the micropipette under controlled pressures and, hence, velocities. Accordingly, the transfer of cytoplasmic particulates from one cell to another by microinjection is possible.

Two interesting examples of cytoplasmic transfer will be described in this report. A volume of cytoplasm, ranging from 500 to 1,000 cubic microns, as measured with the volumetric submicromanipulator, was extracted from an isolated liver cell (frog) with a siliconized micropipette and protected with a fluorocarbon seal. The micropipette was removed from the cell and sealed. It was then reinserted into the same cell and the contents were injected. It was difficult to see where the injected cytoplasmic residue was placed, except that the approximate location was indicated by the small volume of fluorocarbon introduced with the cytoplasm. An important feature of double-seal transfers is that the outer seal is injected into the cell where the fluorocarbon fluid forms a spherical droplet. This technique not only indicates that the injection was accomplished but, in addition, the injected cell is thereby marked for future reference.

Using frog liver cells again, a similar volume of cytoplasm (500 to 1,000 cubic microns) was transferred from one cell to another. The cytoplasm taken from one liver cell and injected into another similar cell was not accompanied by the usual effects noted with the injection of aqueous mixtures. Such a reaction is expected, since there is a close match between the material injected and the cytoplasm of the injected cell. It is impossible to evaluate subtle biochemical changes that may have occurred during the interval between extracting and injecting the sample of cytoplasm. Certainly, the physical consistency has not been appreciably altered. There is no question that other transfers of cytoplasmic particulates can now be accomplished. The important point is that the cytoplasm,

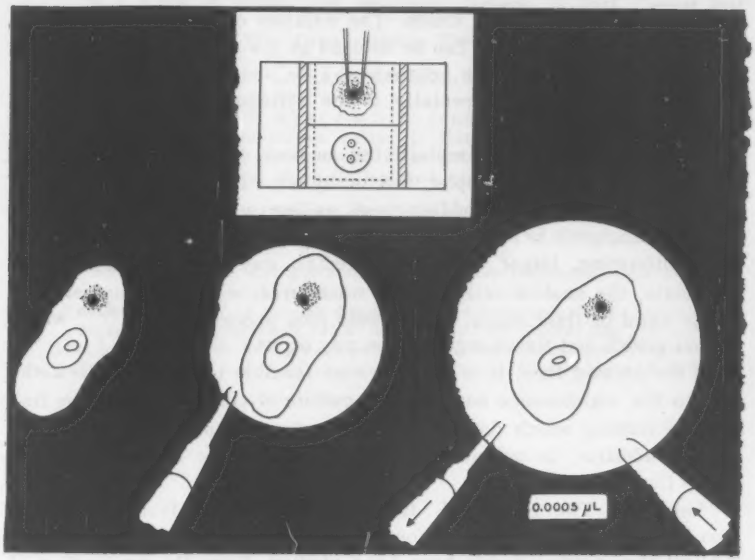

FIGURE 3. **Single-cell cultures. Rectangular insert shows top view of micrurgical moist chamber with two cover glasses and two micropipettes. Upper cover glass has an active culture of the same cells that were isolated in fluorocarbon fluid (lower cover glass). Below are three stages in setting up single-cell cultures. Left cell, marked by a fluorocarbon droplet, is in the nonaqueous medium. In the center, a small volume of culture medium (white) obtained from an active culture was added with a micropipette. On the right, old medium is removed with the left micropipette and fresh medium is added with the right pipette. With this method, volumes as small as 0.0005 μL per cell may be used.**

extracted with a micropipette, is completely surrounded by substances which have no appreciable action on the cytoplasm.

Single-cell cultures. The ability to transfer cytoplasmic particulates from one cell to another will be fruitless unless some means can be provided for maintaining the cells after the operation, in order to evaluate the effects of the transferred particulates. The solution to this problem of cell maintenance is to improve the methods now available for culturing isolated, single cells.

Sanford, Earle and Likely[11] described new procedures for culturing isolated, single cells obtained from various tissues. According to their report, the principal requirement for the successful maintenance of single cells is that the volume of the culture medium be small enough so that the cell can "condition" the medium for its own use. The single cells were isolated in capillary tubes where the volume of the medium was approximately 0.1 μL.

Another procedure, FIGURE 3, involves the addition of small volumes of either fresh or conditioned medium[12] to isolated cells previously

transferred to fluorocarbon fluids. The volumes of medium that may be added to surround the cell can be reduced as low as 0.0005 to 0.01 μL per cell. The handling of such volumes may be done with ordinary microinjection equipment,[13] especially if the syringe is controlled with a micrometer head.[2, 6, 14]

It is also possible to replace the medium whenever necessary by "perfusing" the culture droplet; that is, by removing the old medium with a small micropipette and adding fresh medium with a second, possibly larger micropipette (FIGURE 3). If the single cell "takes," for example, by proliferating, larger volumes of medium may then be added. Subsequently, the nest of cells may be transferred, with a micropipette, to either solid or fluid media, or to media plus sponge matrix,[15,16] where further growth and tissue organization may occur.

At the present time, it is perhaps more feasible to restrict these methods to the maintenance and possible culture of cells obtained from fish or frog tissues which do not require constant temperatures and which may, in addition, be cultured at lower temperatures to avoid evaporation of the fluorocarbons.

Summary. The essential differences resulting from the injection of aqueous and nonaqueous fluids into cells are discussed. Several fluorocarbon fluids have been studied. From the application of these fluids, new procedures for studying the cytochemical properties of subcellular particulates have emerged. The transfer of cytoplasmic fractions from one cell to another is facilitated by first transferring cells from an aqueous environment to the nonaqueous, fluorocarbon fluid. Several precautions for protecting the cytoplasmic particulates during the transfer are described. A new procedure for setting up cultures of single, isolated cells is now available for maintaining cells, following the transfer of cytoplasmic particulates from one cell to another.

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CONFERENCE HELD

SECTION OF PSYCHOLOGY

and

SECTION OF BIOLOGY

PSYCHOTHERAPY AND COUNSELING

Friday, December 3 and
Saturday, December 4, 1954

In this conference five groups selected from disciplines engaged in various types of psychotherapy and counseling presented considered statements on the topics outlined below. Participants were members of the professions of medicine, psychology, social work, the ministry, and counseling and guidance.

The purpose of the conference was to describe the current common and accepted practice by which each of these professions seeks to meet those human needs which can be helped by psychotherapy and counseling; however designated, and what particular training is now being provided. The conference was one step in what may become a series of explorations and interprofessional discussions in which the issues involved in the training and practice of psychotherapy and counseling will be progressively clarified, with a view to the improvement both of the professional services in each profession and the interrelations among these professions.

Preparatory commissions, made up of five to eight members of each of the above professions, have been meeting over the past eight months to review their experience and formulate their views on the following topics. What kinds of persons with what kinds of problems does their profession seek to help by means of psychotherapy or counseling, in individual and group therapy? What methods and practices does their profession use in seeking to help these persons? What kind of training – pre-professional, professional, and supervised experience – is now being prov ded to prepare practitioners for giving such services? Under what circumstances does the profession collaborate with one or another of these professions, and what is the part of each in this collaboration? How are the persons to be trained selected? What are the social expectations and sanctions inherent in the situation in which each profession functions, and how do these influence the practice of the profession? By what methods and techniques does each profession critically examine its own work and seek to advance its professional competence?

At the conference on the evening of December 3 and all day December 4, each of the five commissions presented the consensus and differing views of their members before an invited group, and participated in a discussion of the findings.

Conference Co-Chairmen: Lawrence K. Frank, New York, N. Y.
Rollo May, New York, N. Y.

FRIDAY, DECEMBER 3

8:30 P.M. -

"The Findings of the Commission in Medicine" - Frederick Allen, University of Pennsylvania Medical School, Philadelphia, Pa.

Discussant: Eric Lindemann, Harvard University Medical School, Cambridge, Mass.

General Discussion.

SATURDAY, DECEMBER 4

9:30 A.M. -

"The Findings of the Commission in Psychology" - Nevitt Sanford, Mary Conover Mellon Foundation, Vassar College, Poughkeepsie, N. Y.

Discussants: Robert W. White, Harvard University, Cambridge, Mass.
George A. Kelly, Ohio State University, Columbus, Ohio

General Discussion.

11:00 A.M. -

"The Findings of the Commission in Social Work" - Luther E. Woodward, Community Mental Health Services, New York State Mental Health Commission, New York, N. Y.

Discussants: Lucille Nichol Austin, New York School of Social Work, Columbia University, New York, N. Y.
Helen Harris Perlman, School of Social Administration, University of Chicago, Chicago, Ill.

General Discussion.

2:00 P.M. -

"The Findings of the Commission in Counseling and Guidance" William G. Perry, Jr., Harvard University, Cambridge, Mass.

Discussants: Roland Baxt, Federation Employment and Guidance Service, New York, N. Y.
Philip Zlatchin, New York University, N. Y.

General Discussion.

3:30 P.M. -

"The Findings of the Commission in the Ministry" - The Rev. Wayne E. Oates, Southern Baptist Theological Seminary, Louisville, Ky.

Discussants: The Rev. Paul Johnson, Boston University, Boston, Mass.
Father Noel Mailloux, Center for Research in Human Relations, Montreal, Canada.

5:00 P.M. -
Summary, Lawrence K. Frank, New York, N. Y.

The Section of Psychology and Section of Biology provide conferences for active workers in the special fields of Psychology and Biology.

Attendance is limited to those invited to participate in these conferences and to interested Members of the Academy.

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Elected December 13, 1954

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